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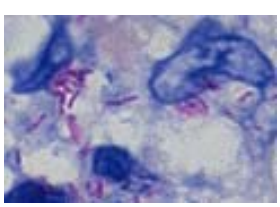
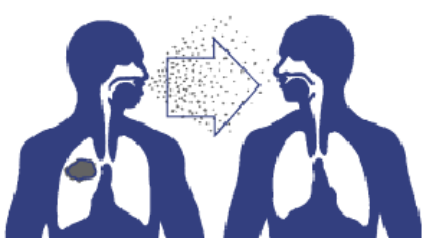
BIOMÉRIEUX

Customer Training Manual: Mycobacterial Testing With BacT/ALERT® Systems and Media

LCR # 09-0112 PN 60-00467-0





Global Customer Support GCS K5 GCS TM 0383 31JAN09

Summary

MYCOBACTERIAL TESTING WITH BACT/ALERT® SYSTEMS AND MEDIA

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PURPOSE: This manual contains theory, troubleshooting, Best Practices and tips to remember. It is intended for use as a support and instructional guide for customers and bioMérieux Applications Specialists in performing mycobacterial testing with BacT/ALERT® Systems and Media. After completing this manual, one should be competent in basic theory of sample processing and daily workflow of the BacT/ALERT® MP and MB culture bottles. This manual should be used in conjuction with the referenced User Manuals and appropriate media Instructions for Use.

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REFERENCES:

BacT/ALERT® User Manual Version B.25, PN 514277-2EN1 (12/2005)
BacT/ALERT® Instructions for Use, July 2008
Footnote references as cited in document

BACKGROUND INFORMATION: Mycobacteria in our World

OBJECTIVES: To understand the importance of Mycobacterial testing

DISCUSSION

Tuberculosis remains a major global public health problem. The World Health Organization estimates that 9 million new cases and 2 million deaths are directly attributable to the disease each year.[1] Tuberculosis has been cited as the leading cause of death in many resource-poor and developing countries.[2] Infections caused by *M. tuberculosis* complex organisms are classically pulmonary. If control of tuberculosis is not further strengthened in the future, the World Health Organization estimates that between 2000 and 2020, nearly one billion people will be newly infected, 200 million people will become sick, and another 35 million people will die from tuberculosis.[2]

Mycobacteria other than the tubercle bacillus (MOTT) are referred to here as nontuberculosis mycobacteria (NTM). The majority of these isolates require incubation at 35 to 37°C except where noted. These organisms are most commonly found in soil and water and have been implicated as opportunistic pathogens in patients with underlying lung disease, immunosuppression or percutaneous trauma.[3]

NTM associated with pulmonary disease are: *M. avium complex* (MAC), *M. kansasii, M. asiaticum, M. fortuitum complex* (rarely pulmonary)*, M. szulgai, M. malmoense, M. shimoidei* (grows well at 45°C)*, M. celatum*, and *M. xenopi* (optimum temperature of 40-42°C). *M. marinum* and *M. ulcerans*, which require incubation at 30°C, are NTM associated with cutaneous infections. NTM producing other types of infection (e.g. disseminated disease, lymphadenitis, osteomyelitis, etc.) are: MAC, *M. kansasii, M. scrofulaceum, M. fortuitum* complex (*M. chelonae* isolates generally require incubation at 30°C), *M. szulgai, M. simiae, M. genavense* (requires Mycobactin J and 8 to 12 weeks incubation)*, M. celatum*, and *M. haemophilum* (requires addition of hemin and incubation at 30°C).[2, 4]

"Classic examples of NTM contamination include the isolation of *M. gordonae*, *M. mucogenicum*, or *M. terrae* complex from sputum on culture. These species are common in tap water and almost never cause chronic lung disease. *M. mucogenicum*, for example, has been cultured from almost half of the samples of drinking water and ice investigated in the United States; fewer than 5% of the clinical isolations are considered medically significant. Likewise, *M. gordonae* rarely is associated with clinical disease, and then almost exclusively in the setting of severe

[1] Global tuberculosis control: surveillance, planning, financing. WHO report 2007. Geneva, World Health Organization (WHO/HTM/TB/2007.376).
[2] Pfyffer GE, Brown-Elliott BA, and Wallace RJ, Jr.: Mycobacterium, in Murray PR, Baron EJ, Pfaller MA, et al (eds): *Manual of Clinical Microbiology*, ed 8. Washington, DC, American Society for Microbiology, 2003, pp 532-559.
[3] Roberts GD, Koneman EW, Kim YK: Mycobacterium in Balows A, Hausler WJ Jr, Herrmann KL, et al (eds): *Manual of Clinical Microbiology*, ed 5. Washington, DC, American Society for Microbiology, 1991, pp 304-339.
[4] CLSI. *Laboratory Detection and Identification of Mycobacteria*; Approved Guideline. CLSI document M48-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

immunosuppression. Many new species, including *M. botniense*, *M. cookii*, *M. chlorophenolicum, M. frederiksbergense*, *M. hodleri,* and *M. murale*, have recently been identified from environmental samples but not yet identified as human pathogens. *M. celatum* is another organism frequently isolated in settings in which it is not considered clinically significant, though the pathogenicity seems higher than many species of NTM, especially in patients with acquired immune deficiency syndrome (AIDS).

Some species are associated with specific disease syndromes, and their isolation in that setting is often highly significant. Table 1, below, shows examples of mycobacterial species and clinical conditions in which they often play a significant role."[1]

Table 1. Examples of Mycobacterial Species and Clinical Conditions

NTM species	Clinical setting	Sample
M. abscessus or *M. avium* complex (MAC)	Cystic fibrosis (CF) or bronchiectasis in older Caucasian women	Respiratory
M. fortuitum	Esophageal achalasia and chronic regurgitation, or history of mineral oil ingestion	Respiratory
M. marinum	Extremity lesion after fish tank or other marine exposure	Biopsy/aspirate
M. kansasii or MAC	Hairy-cell leukemia, advanced HIV disease	Blood, bone marrow
M. chelonae, M. abscessus, or *M. haemophilum*	Disseminated skin lesions in patients on chronic immunosuppressive drugs, eg, corticosteroids	Biopsy, aspirate
M. chelonae, M. mucogenicum, M. fortuitum, or *M. abscessus*	Central venous catheter, prosthetic device, surgical wound, injection site, or other local trauma	Biopsy, aspirate

Table is from CLSI M48-A

Mycobacteria are aerobic, nonsporeforming, nonmotile, acid-fast bacilli which have slow to very slow growth rates; generation times of species vary from 2 to >20 hr. depending upon the species.[2] Growth of *M. tuberculosis* using traditional mycobacterial media can take two to eight weeks or longer.[3] CDC (Centers for Disease Control) recommendations suggest that a liquid culture medium be used, along with solid media, for primary culture. "It is recommended that whenever possible, liquid medium should be used to assure better and more rapid results. The addition of solid medium along with a liquid medium maximizes the recovery, provides an opportunity to look at colony morphology that is not possible in liquid media, allows for detection of mixed mycobacterial infections and serves as a backup when liquid culture is contaminated. An increase of 4 to 6% for mycobacterial isolation has been reported by the addition of an LJ slant along with a liquid medium; while adding a liquid medium along with an LJ medium is reported to increase the recovery rate significantly (15 to 30%)."[4] The liquid-culture method is needed to provide more rapid detection, growth, and susceptibility results for *M. tuberculosis*.[5, 6]

[5] Essential components of a tuberculosis program: recommendations of the Advisory Council for the Elimination of Tuberculosis. *MMWR* 1995;44 (No. RR-11):13.

The MB/BacT® and the BacT/ALERT® 3D Mycobacteria Detection Systems, used in conjunction with the BacT/ALERT® MP culture bottle, provide both a microbial detection system and a culture media with suitable nutritional and environmental conditions to recover mycobacterial species commonly isolated from patient specimens other than blood. The MB/BacT® Antibiotic Supplement is intended to reduce the incidence of break-through contamination due to bacteria that may survive the decontamination/ concentration process. MB/BacT® Antibiotic Supplement must be added to BacT/ALERT® MP culture bottles prior to inoculation of all non-sterile specimens. For recovery of mycobacteria present in sterile specimens, only the MB/BacT® Reconstitution Fluid should be added to the BacT/ALERT® MP culture bottles. MB/BacT® Reconstitution Fluid contains components that are necessary to ensure optimal growth of mycobacteria present in the patient sample. Inoculated bottles are placed into the instrument where they are incubated and continuously monitored for the presence of mycobacteria that will grow in the BacT/ALERT® MP culture bottle. At the time of detection, approximate colony forming units (CFUs) per ml are 10^6-10^7.[7]

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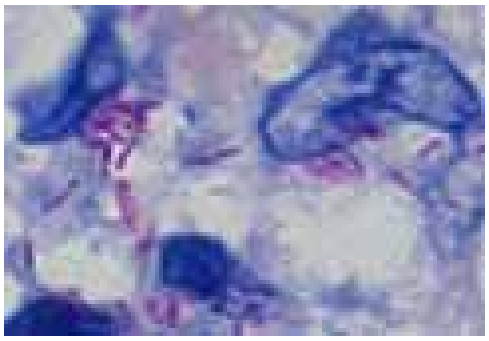
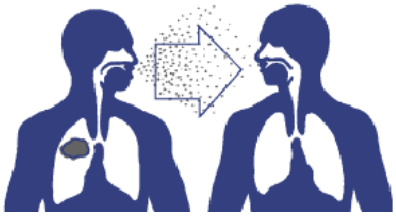


[6] Tenover FC, Crawford JT, Huebner RE, et al: The resurgence of tuberculosis: is your laboratory ready? *J Clin Micro* 31(4): 767-770, 1993.
[7] bioMérieux, BacT/ALERT® MP Instructions for Use, July 2008.

EXERCISE SESSION (See Appendix I for answers)

1. With 2 million deaths a year, what is the leading cause of death in many resource-poor and developing countries?
2. What are MOTT?
3. Why is a solid media recommended when using liquid media culture systems?

TIPS to REMEMBER



Mycobacteria are aerobic, nonsporeforming, nonmotile, acid-fast bacilli which have slow to very slow growth rates; generation times of species vary from 2 to >20 hours and growth of *M. tuberculosis* using traditional mycobacterial media can take two to eight weeks or longer.

Liquid medium should be used to assure better and more rapid results. The addition of solid medium along with a liquid medium maximizes the recovery, provides an opportunity to look at colony morphology that is not possible in liquid media, allows for detection of mixed mycobacterial infections and serves as a backup when liquid culture is contaminated.

THEORY OF OPERATION

OBJECTIVES: To understand how the BacT/ALERT® Systems operate

DISCUSSION: Theory of Operation

The MB/BacT® (Classic) and BacT/ALERT® 3D Mycobacteria Detection System are totally automated test systems for incubating and monitoring culture bottles for microbial growth. Both systems use the same technology to read and determine positive and negative cultures. The system consists of incubators, data management computers, growth media bottles and reagents. Mycobacteria behave like most other bacteria with respect to carbohydrate metabolism, energy production, and the biosynthesis of low weight metabolites. Glycerol and oleic acid were selected as primary carbon sources in the BacT/ALERT® MP and BacT/ALERT® MB culture bottles because of their ability to maximize the amount of carbon dioxide (CO_2) generated by mycobacteria.

Once ingested, glycerol and oleic acid are converted to Acetyl-CoA and are oxidized through the Krebs or Tricarboxylic Acid Cycle (TCA). Carbon Dioxide (CO_2) and free electrons are the major metabolic byproducts of oxidation. Although mycobacteria divide slowly, the level of CO_2 generated is similar to the levels seen in most common bacteria.

The MB/BacT® and BacT/ALERT® 3D Mycobacteria Detection Systems utilize a colorimetric sensor and reflected light to monitor the amount of CO_2 dissolved in the culture medium. If microorganisms are present in the test sample, they produce CO_2 as they metabolize the substrates in the culture medium.

The liquid emulsion sensor (LES) is impermeable to most ions including hydrogen ions, and to components of media and whole as well as degraded blood. It is freely permeable to CO_2. As CO_2 diffuses across the membrane and dissolves in the water contained in the sensor, free hydrogen ions are generated:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3$$

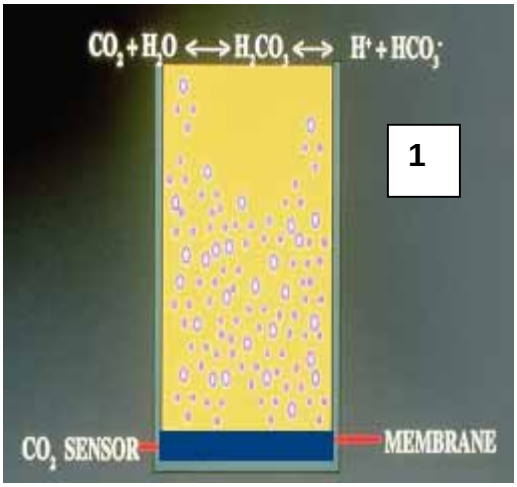
Free hydrogen ions interact with the indicator in the sensor. As CO_2 is produced, the concentration of hydrogen ions increases and the pH falls causing the sensor to change to lighter green or yellow.

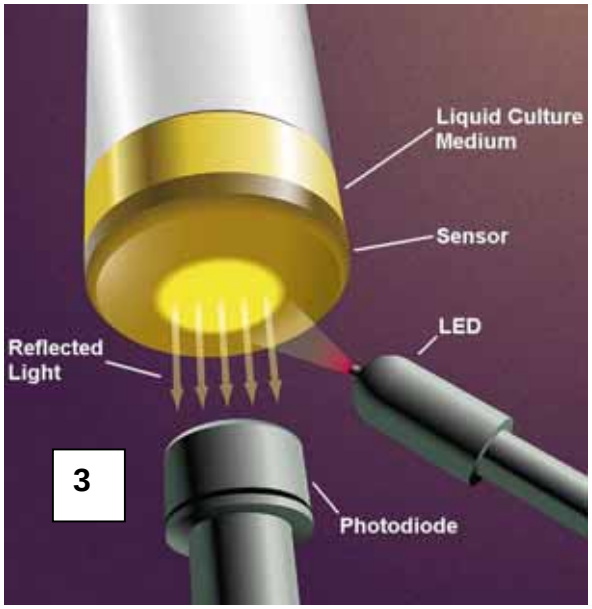
A Light Emitting Diode (LED) projects light onto the sensor. The light reflected by the sensor is measured by a photodetector, and as more CO_2 is generated, more red light is reflected (lighter colors reflect more light than darker colors).

The photodiode converts the reflected light into an electrical signal that is measured every 10 minutes and plotted every hour as a point on a graph. Mathematical algorithms analyze the readings and slope of the curve over time to determine

positives and negatives. A representative graph for most bacteria reflects a typical growth curve with a lag phase, log phase, and stationary phase of growth. The MB/BacT® and BacT/ALERT® 3D Mycobacteria Detection Systems monitor bottles using specific mycobacterial process bottle and mycobacterial blood bottle algorithms. These algorithms employ bacterial and mycobacterial algorithms for the first 4 days of incubation and thereafter use the very sensitive mycobacterial algorithm only. In addition to detecting rapid growers, the bacterial algorithms detect any breakthrough bacterial contamination during the first four days of incubation. Each sample is monitored independently, with its own established baseline. bioMérieux recommends a test protocol of 42 days for all mycobacterial bottles.

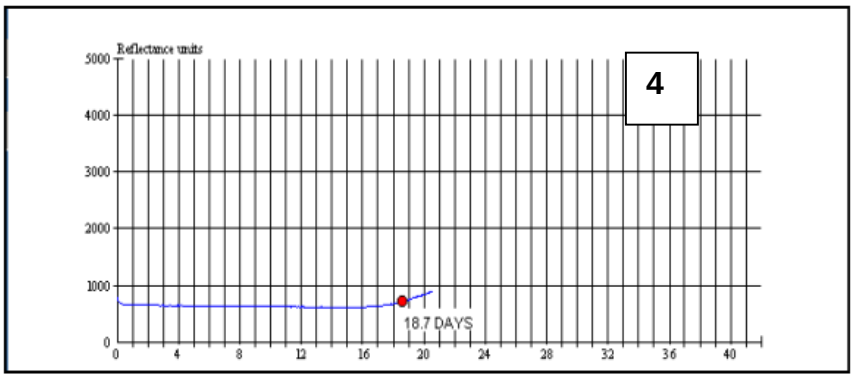
TRAINING SESSION: Theory of Operation





1. Organisms grow and produce CO_2
2. The bottle sensor lightens in color
3. Reflectance is measured and analyzed
4. A graph of the growth curve is produced

Mycobacterium tuberculosis



EXERCISE SESSION (See Appendix I for answers.)

1. What metabolites were chosen to enhance CO_2 production during Mycobacterial metabolism?
2. What technology is used to monitor the production of CO_2?
3. Why does the sensor change colors from dark to light?

TIPS to REMEMBER: CO_2 is produced by growing organisms and results in a lowered pH. The sensor changes color to lighter green or yellow. A representative graph for most bacteria reflects a typical growth curve with a lag phase, a log phase, and a stationary phase of growth. Mathematical algorithms in the software analyze the readings and slope of the curve over time to detect log phase growth and determine positive and negative results.

BacT/ALERT® MYCOBACTERIA PRODUCTS OVERVIEW

OBJECTIVES: To be familiar with BacT/ALERT® Mycobacterial Products

DISCUSSION: BacT/ALERT® MP System

Reagent	Product description	Product number	Case Size and Storage	Inoculum size	Media Contents
BACT/ALERT® MP culture bottle (Plastic)	Red cap 10 ml	**259797**	100 bottles per case 2-8° C in dark	0.5 ml	Middlebrook 7H9 broth, Pancreatic Digest of Casein, Bovine Serum Albumin, Catalase in purified water with an atmosphere of CO_2, nitrogen and oxygen under vacuum.
MB/BacT® Antibiotic Supplement kit contains 2 reagents for MP culture bottle:		**259760**	100 tests per kit	0.5 ml for non-sterile specimens	Kit contains 5 vials of lyophilized antibiotic power and 5 vials of reconstitution fluid.
Reagent #1 is MB/BacT® Antibiotic Supplement	Lyophilized antibiotic cake		5 vials per kit 2-8° C in dark	0.5 ml into MP culture bottle for non-sterile specimens	Lyophilized cake of 6 antibiotics: Amphotericin B, Azlocillin, Nalidixic Acid, Polymyxin B, Trimethoprim, and Vancomycin.
Reagent #2 is MB/BacT® Reconstituiton Fluid	Red fluid 15 ml		5 vials per kit 2-8° C in dark	rehydrate reagent #1 with 10 ml of reagent #2 0.5 ml into MP culture bottle for sterile specimens	Contains oleic acid, Glycerol, Amaranth and Bovine Serum Albumin in purified water.growth factors. Use 10 ml to rehydrate one antibiotic vial. Use remaining 5 ml for sterile body fluids in MP culture bottle.
BacT/ALERT® Reseal	Clear	**259787**	100/ box Room Temp		Clear plastic snap cap used to reseal stopper after removing bottle crimp for needless inoculation of MP bottle.

NOTE: Prior to use, the BacT/ALERT® MP culture bottles should be examined for evidence of damage or deterioration (discoloration). Bottles exhibiting evidence of damage, leakage, or deterioration should be discarded. The media in undisturbed bottles should be clear. Do not use a bottle containing media that exhibits turbidity, a yellow sensor, or excess gas pressure; these are signs of possible contamination. Also inspect all MB/BacT® Antibiotic Supplement Kit vials for evidence of damage or contamination. Do not use MB/BacT® Reconstitution Fluid vials exhibiting turbidity.

TRAINING SESSION: BacT/ALERT® MP culture bottles, MB/BacT® Antibiotic Supplement Kit and BacT/ALERT® Reseals

BacT/ALERT® MP culture bottles – Plastic – 100 bottles per case

BacT/ALERT® Reseals PN 259787

Reagent 1: Lyophilized antibiotic cake
Reagent 2: Reconstituiton fluid

NOTE: Rehydrate powder with 10 ml reconstitution fluid and discard unused portion 7 days after rehydration.

EXERCISE SESSION (See Appendix I for answers.)

1. How many BacT/ALERT® MP culture bottles are in one case?
2. What are the storage conditions for the BacT/ALERT® MP culture bottles and the MB/BacT® Antibiotic Supplement Kit?
3. How many bottles can be prepared with one box of the MB/BacT® Antibiotic Supplement Kit?

TIPS to REMEMBER:

MB/BacT® Reconstitution Fluid vials contain 15 ml. The MB/BacT® Antibiotic Supplement vials are reconstituted with 10 ml of MB/BacT® Reconstitution Fluid, leaving 5 ml for sterile body fluid bottles. Either 0.5 ml of the reconstituted antibiotic

cake (non-sterile, decontaminated specimens) or 0.5 ml of reconstitution fluid (sterile specimens) must be added to bottles to assure the presence of Mycobacterial growth factors.

BacT/ALERT® MP culture bottles must be loaded into cabinets (BacT/ALERT® Classic) or drawers (BacT/ALERT® 3D) that are designated "MB" cabinets or drawers. This assures that the correct mycobacterial culture bottle algorithm is assigned to analyze the bottle readings.

DISCUSSION: BacT/ALERT® MB System

Reagent	Product description	Product number	Case Size and Storage	Inculum size	Media Contents
BACT/ALERT® MB Culture Bottle (Glass)	Black cap 29 ml	**251011**	25 bottles per case Room Temperature (15-30° C) in dark	3 - 5 ml whole blood	Middlebrook 7H9 broth, Pancreatic Digest of Casein, Glycerol, SPS, in purified water with an atmosphere of CO_2, nitrogen and oxygen under vacuum.
MB/BacT® Enrichment Fluid for the MB bottle	Black 5.5 ml	**259877**	25 tests per kit Room Temperature (15-30° C) in dark	1.0 ml into MB culture bottle	Bovine Serum Albumin, Sodium Chloride, Oleic Acid, Saponin in purified water. Enrichment fluid must be added to the MB bottle within a 24 hour window of inoculation of specimen. Kit contains 5 vials.
BACT/ALERT® Blood Collection Adapter Cap / Insert for filling of blood in BACT/ALERT® MB culture bottles	Clear	**210361 / 210362**	120/ case 60/ case Room Temp		Cap for use with butterfly collection set for direct draw of blood. Insert allows blood to be filled into vacuum collection tubes.

NOTE: Prior to use, the BacT/ALERT® MB culture bottles should be examined for evidence of damage or deterioration (discoloration). Bottles exhibiting evidence of damage, leakage, or deterioration should be discarded. The media in undisturbed bottles should be clear. Do not use a bottle containing media that exhibits turbidity, a yellow sensor, or excess gas pressure; these are signs of possible contamination. Also inspect all MB/BacT® Enrichment Fluid vials for evidence of damage or contamination. Do not use MB/BacT® Enrichment Fluid vials exhibiting turbidity.

TRAINING SESSION: BacT/ALERT® MB culture bottles, MB/BacT ® Enrichment Fluid, BacT/ALERT® Blood Collection Adaptor Caps and Inserts

BacT/ALERT® MB culture bottles – GLASS with plastic sleeve!

25 bottles per case

MB/BacT® Enrichment Fluid

Inoculate 3-5 ml blood into bottle:
•Direct with needle and syringe
•Direct using adapter cap
–cap PN/ 210361
–insert PN/ 210362
•Sterile transport tube: heparin (sodium or lithium) or SPS (sodium polyanetholesulfonate) tube

EXERCISE SESSION (see Appendix I for answers)

1. How many BacT/ALERT® MB culture bottles are in one case?
2. What are the storage conditions for the BacT/ALERT® MB culture bottles and the MB/BacT® Enrichment Fluid?
3. How many bottles can be prepared with one box of MB/BacT® Enrichment Fluid?

TIPS to REMEMBER:

BacT/ALERT® MB culture bottles are GLASS with a plastic sleeve! DO NOT attempt to send these bottles through a pneumatic tube system!

Because the tops of these bottles are larger than the clinical plastic BacT/ALERT® culture bottles, the adaptor cap for this bottle is also larger and specific for this bottle.

BacT/ALERT® MB culture bottles may be loaded into any cabinets (BacT/ALERT® Classic) or drawers (BacT/ALERT® 3D). The BacT/ALERT® MB culture bottles may incubate in shaking or non-shaking cabinets and drawers. However, these bottles may NEVER be loaded anonymously! Scanning the bottle barcode directs the software to assign a specific mycobacteria blood bottle algorithm to analyze the bottle readings in which the “Delta” portion of the mycobacterial algorithm is disabled.

INTRODUCTION TO PROCESSING: PROCEDURAL STEPS AND BEST PRACTICES

OBJECTIVES: To understand the processing procedure and to know how to obtain the best sample possible for patient testing.

DISCUSSION: BACT/ALERT® MP CULTURE BOTTLE PROCEDURE

The BacT/ALERT® MP System includes the BacT/ALERT® MP culture bottles and the MB/BacT® Antibiotic Supplement Kit (MAS). The MB/BacT® Antibiotic Supplement Kit includes MB/BacT® Reconstitution Fluid and MB/BacT® Antibiotic Supplement. The reconstituted antibiotic supplement is intended to reduce the incidence of breakthrough contamination due to bacteria that may survive the decontamination/digestion process. The MB/BacT® Reconstitution Fluid contains components necessary to ensure optimal growth of mycobacteria present in the patient sample and must be added to all bottles to ensure growth of mycobacteria. The red color of the reagent allows the user to know that this reagent has been added to the BacT/ALERT® MP culture bottle. Inoculation of solid media and the BacT/ALERT® MP culture bottle is recommended for optimal recovery of mycobacterial organisms from specimens.

The recommended decontamination method is N-Acetyl-L-Cysteine-Sodium Hydroxide (final or working concentration of 2%) for non-sterile specimens. For sterile body fluids (except whole blood), 0.5 ml sample is inoculated directly into the BacT/ALERT® MP culture bottle with 0.5 ml of the MB/BacT® Reconstitution Fluid. Non-sterile specimens (including fluids that contain ANY bacteria other than mycobacteria) must be decontaminated and 0.5 ml of pellet (pH neutral) inoculated into the bottle with 0.5 ml of reconstituted antibiotic supplement. The red colored MB/BacT® Reconstitution Fluid provides nutrients and growth factors and is required. The MB/BacT® Antibiotic Supplement is rehydrated with MB/BacT® Reconstitution Fluid. All BacT/ALERT® MP culture bottle media should have a light pink color prior to inoculation, indicating that either reconstitution fluid or reconstituted antibiotic supplement has been added. The reconstituted antibiotic supplement is not intended to overcome lack of good sterile technique or grossly contaminated specimens. Proper specimen handling and good laboratory techniques must be practiced to avoid a high contamination rate. Drug resistant strains of bacteria may not be inhibited.

THEORY AND BEST PRACTICES: MYCOBACTERIA SPECIMEN PROCESSING

Liquefaction: Many specimens submitted for mycobacterial isolation contain mucus such as sputum and gastric lavage. Mycobacteria, as well as contaminating flora, are often present but trapped within the mucus. Liquification is achieved by adding chemicals which, when vortexed with the specimen, break down the mucus and release the organisms.

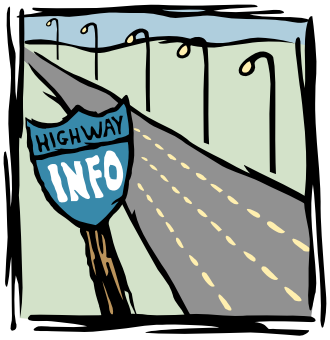
"Several agents can be used to liquefy a clinical specimen, including NALC, dithiothreitol (sputolysin), and enzymes. In most procedures, liquification (release of the organisms from mucin or cells) is enhanced by vigorous mixing with a vortex type of mixer in a closed container. Following mixing, the container should be allowed to stand for 15 minutes before opening, to prevent the dispersion of fine aerosols generated during mixing."[1]

Decontamination: "Most specimens received for mycobacterial culture contain various amounts of organic debris and a variety of contaminating, normal, or transient bacterial flora. A chemical decontamination process usually effectively kills the contaminants while allowing

[1] Forbes BA, Sahm DF, Weissfeld AS. *Diagnostic Microbiology*, St. Louis, Missouri, Mosby, 1998. p 726

recovery of the mycobacteria. The high lipid content of the Acid Fast Bacilli cell wall makes the mycobacteria more resistant to both acid and alkaline decontaminating agents. Strict adherence to the timed killing period is necessary to maximize recovery."[2]

"Sodium hydroxide, the most commonly used decontaminant, also serves as a mucolytic agent but must be used cautiously because it is only somewhat less harmful to tubercle bacilli than to the contaminating organisms. The stronger the alkali, the higher its temperature during the time it acts on the specimen, and the longer it is allowed to act, the greater will be the killing action on both contaminants and mycobacteria."[3]



TECHNICAL TIPS:

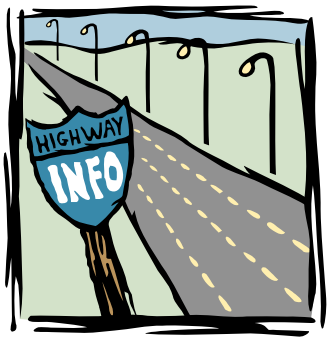
1. If you are experiencing higher than expected false positives or breakthrough contamination, use no more than 5 mL of sample per tube. To avoid discarding sample, split larger samples into multiple aliquots and process separately. Pellets may be combined at end of processing for inoculation into one BacT/ALERT® MP culture bottle. If less than 5 mL of sample is available, add sterile 0.067 M phosphate buffer pH 6.8 or sterile water to bring the volume up to the 5 mL mark.
2. Use individual pour tubes to prevent the addition of excess decontamination reagent and to avoid the possibility of cross-contamination between specimens.

"Most commonly, a combination liquefaction-decontamination mixture is used. These agents have no direct inhibitory effect on bacterial cells; however, their use permits treatment with lower concentrations of sodium hydroxide, thereby indirectly improving the recovery of mycobacteria. Specimens submitted for the culture of mycobacteria should be processed as soon as possible. The best yield of mycobacteria may be expected to result from the use of the mildest decontamination procedure that sufficiently controls contaminants. Strict adherence to specimen processing procedures is mandatory to ensure survival of the maximal number of mycobacteria."[4]

"All currently available digesting/decontaminating agents are to some extent toxic to tubercle bacilli; therefore, to ensure the survival of the maximum number of bacilli in the specimen, the digestion/decontamination procedure must be precisely followed. In order for enough tubercle bacilli to survive to give a confirmatory diagnosis, it is inevitable that a proportion of cultures will be contaminated by other organisms…It is also important to note that a laboratory which experiences no contamination is probably using a method that kills too many of the tubercle

[2] Cernoch PL, Enni RK, Saubolle MA, Wallace RJ, Weissfeld AS (ed): Laboratory Diagnosis of the Mycobacterioses; *Cumulative Techniques and Procedures in Clinical Microbiology.* Washington D.C., American Society of Microbiology 1994. p 8
[3] Pfyffer GE, Brown-Elliott BA, Wallace RJ: Mycobacterium: General Characteristics, Isolation, and Staining Procedures, in Murray PR, Baron EJ, Pfaller MA, et al (eds): *Manual of Clinical Microbiology,* ed 8. Washington, DC, American Society for Microbiology, 2003, p 544.
[4] Cernoch PL, Enni RK, Saubolle MA, Wallace RJ, Weissfeld AS (ed): Laboratory Diagnosis of the Mycobacterioses; *Cumulative Techniques and Procedures in Clinical Microbiology.* Washington D.C., American Society of Microbiology 1994. p 8

bacilli."[5] "The acceptable range is 3 to 5% with media without antimicrobial agents. A contamination rate significantly less that 3% in the non-antimicrobial-agent-containing media suggests overly harsh decontamination. Greater than 5% growth suggests inadequate decontamination or incomplete digestion."[6] Overly harsh decontamination may damage or destroy mycobacteria in samples, resulting in lower detection rates or prolonged time to detection.



TECHNICAL TIPS:

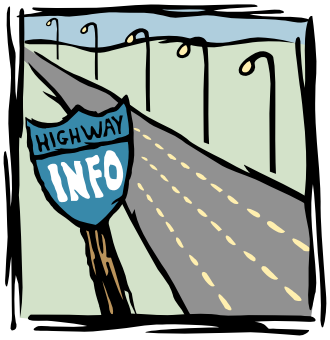
1. **Use individual pour tubes** containing precise volumes of decontamination reagent to prevent the addition of excess reagent that will require pH neutralization later and to avoid the possibility of cross-contamination between specimens.
2. Vortex for a minimum of 20 seconds up to a maximum of 30 seconds. **Use a timer!**
3. Make sure to **invert the tube** a few times during the vortexing process to insure decontamination of all surfaces of the specimen tube.
4. Extremely mucoid or bloody specimens may require additional NALC powder for complete digestion if you are using the NALC-NaOH method.
5. Observe timing of decontamination step. Longer exposure times cause more destruction of the mycobacteria. Do not process too many samples at one time (>20).
6. When adding the neutralization buffer to stop the action of the digestion agent, pour from an individual pour tube to avoid the possibility of cross-contamination between specimens. **NOTE: Ideally, the volume of decontamination reagent and neutralization buffer used will not vary from tube-to-tube.**
7. **Add buffer to the 50 mL mark on the centrifuge tube** to maximize buffering capability of this step.
8. Mix by inversion to ensure all digestion agent is neutralized within the sample tube.
9. To control breakthrough contamination rates after first assuring that procedure, vortexing and sterile technique are adequate, make small incremental increases of 0.5% in the decontamination reagent (NaOH) concentration. **Verify that a pH of 6.8 to 7.5 is maintained in the final sample pellet with each change in NaOH concentration.**

[5] Kantor IN, Kim SJ, Frieden t, et al. 1998. Laboratory Services in Tuberculosis Control: Culture Part lll. WHO/TB/98.258. p 37

[6] Della-Latta P (ed): Mycobacteriology and Antimycobacterial Susceptibility Testing, in Isenberg HD (ed). *Clinical Microbiology Procedures Handbook*, vol 2. Washington, DC, ASM Press; 2004 sect 7.1.2.2

Concentration: Mycobacteria are often present in clinical specimens in very small numbers; therefore it is essential to concentrate by centrifugation before inoculating to cultures. High centrifugation speeds create heat that will kill mycobacteria, especially in the presence of chemicals. Therefore optimal results are obtained with a refrigerated centrifuge. "The centrifuge must be fast enough to attain a relative centrifugal force (RCF) of 3000 x g. If the RCF is not high enough, many tubercle bacilli remain in suspension following centrifugation and are poured off with the discarded supernatant fluid. Recent studies have shown that 3000 x g for 15 minutes would sediment 95% of mycobacteria in a digested sputum specimen. The specific gravity of tubercle bacilli ranges from 1.07 to 0.79, making centrifugal concentration of specimens ineffective if the RCF is not 3000 x g."[7]

The relative centrifugal force (RCF or *g*) at a given radius is a function of the revolutions per minute (rpm) in a centrifuge. Use the nomogram on the following page to determine the relative centrifugal force, the revolutions per minute, or the radius when only two of these three variables are known. To find the unknown value, draw a straight line using a ruler through the two known values. Read the unknown value from the point of intersection on the column corresponding to that variable. For example, if the radius of the centrifuge to be used is 160 mm, one would need to spin at 5000 rpm to achieve the minimum of 3000 x *g*.



TECHNICAL TIPS:

1. Check the radius and rpms of the centrifuge to **be sure 3000 X g is achieved during concentration** of the sample.
2. **Pour off supernatant completely** into a splash-proof discard container. This allows for better pH control after resuspension of the pellet.
3. Carefully wipe the lip of the tubes with individual gauze pad dampened with disinfectant – **avoid getting the disinfectant into the sample tube.**
4. **Re-suspend the pellet in 1-2 mL of sterile 0.067 M phosphate buffer, pH 6.8,** using individual sterile transfer pipettes. Lower resuspension volumes (less dilution) increase the numbers of organisms in the 0.5 mL sample to be inoculated into the bottle.
5. Do not put other additives, such as albumin, into the sample pellet prior to inoculation.
6. Periodically check the final pH of the re-suspended pellet. It should be between pH 6.8 and 7.5. Higher pH may cause false positives, and be detrimental to the mycobacteria, delaying the time to detection. Lower pH may cause false positives.

[7] Kantor IN, Kim SJ, Frieden t, et al. 1998. Laboratory Services in Tuberculosis Control: Culture Part lll. WHO/TB/98.258. p 38

Relative Centrifugal Force Nomogram:

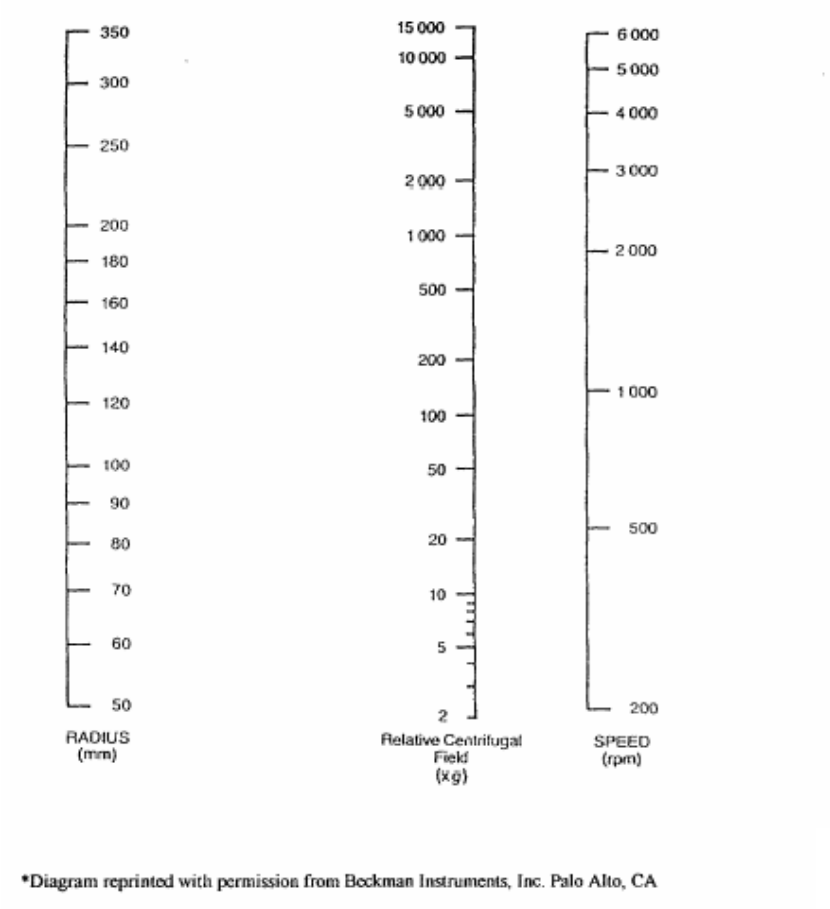
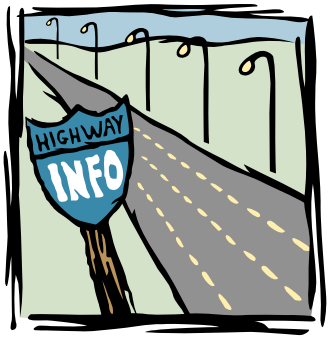


*Diagram reprinted with permission from Beckman Instruments, Inc. Palo Alto, CA

Inoculation: "It is generally accepted that the use of a liquid medium in combination with at least one solid medium is essential for good laboratory practice in the isolation of mycobacteria. Addition of a solid medium is advantageous for the detection of strains which occasionally do not grow in liquid medium, aids in the detection of mixed mycobacterial infections, and can serve as a back-up for broth cultures, if contaminated. All positive cultures, even if identified directly from the broth, must be subcultured to solid media to detect mixed cultures and to correlate direct identification results with colony morphology."[8]

It is important to resuspend the concentrated pellet in as little liquid as possible to maintain the largest numbers of organisms to inoculate cultures. Larger numbers of organisms lessen the time to detection in the BacT/ALERT® culture bottle and enhance smear results.

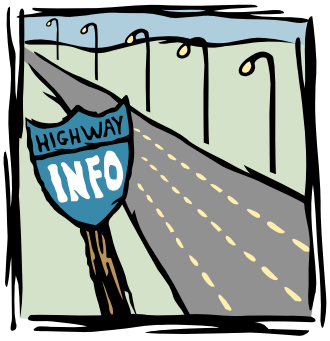
[8]Pfyffer GE, Brown-Elliott BA, Wallace RJ: Mycobacterium: General Characteristics, Isolation, and Staining Procedures, in Murray PR, Baron EJ, Pfaller MA, et al (eds): *Manual of Clinical Microbiology,* ed 8. Washington, DC, American Society for Microbiology, 2003, p 549.



TECHNICAL TIPS:

1. **Use individual disposable alcohol prep pads** to clean the tops of all reagent or culture bottles before each entry into the bottle.
2. **Add no more than 0.5 mL of MAS** (Mycobacterial Antibiotic Supplement) to each BacT/ALERT® MP culture bottle. Add 0.5 mL of MB/BacT® Reconstitution Fluid alone to the culture bottles for sterile body fluids. Invert to mix MAS, sample and broth.
3. **Note: Increasing the amount of MAS added to the bottle will increase false positives and/or may increase time to detection of mycobacteria. At neutral pH, 0.5 mL of MAS should control most breakthrough contamination in properly digested/decontaminated samples. Re-suspend lyophilized MAS with precisely 10 mL of reconstitution fluid.**
4. When using syringes for obtaining sample from the tube for inoculation into the bottles, **use needles long enough to reach the bottom of the tube.** This aids in avoiding contamination and cross-contamination of samples from gloved hands. Do not use tuberculin syringes and needles, for example, because the needles are too short to reach the bottom of the tube. Use larger bore needles to avoid sampling errors. 18-22 gauge needles are OK, while 23 gauge may be too small.
5. **Decant supernatant immediately** after centrifugation to avoid excessive contact time at alkaline pH and minimize the possibility of the pellet becoming loose.
6. **Inoculate concentrates to media as soon as possible**, never leave overnight. Although buffered, the final concentrate may be slightly alkaline which will destroy mycobacteria in time. The media in the BacT/ALERT® culture bottle serves to further neutralize the inoculum.
7. **Before removing bottles from the biological Safety Cabinet, clean the tops of the bottles with a tuberculocidal agent**.
8. **CAUTION: Be sure this agent is compatible with the polycarbonate BacT/ALERT® culture bottles.**

Although N-Acetyl-L-Cysteine-Sodium Hydroxide is the digestion-decontamination reagent recommended by bioMérieux, there are many digestion-decontamination methods in use for Mycobacterial testing around the world. Most of these methods are successfully employed by BacT/ALERT® customers. However, Zephiran-trisodium phosphate and Cetylpyridinium chloride possess mycobacteriostatic properties and should not be used with any liquid broth system because the chemicals cannot be eliminated by washing or by buffering steps.[9] Regardless of the method used, strict adherence to the recommended procedure assures consistent and accurate results.



Regardless of the method used, the most critical steps are:

1. Decant liquid completely after centrifugation.
2. Re-suspend the pellet in sterile 0.067M phosphate buffer, pH 6.8.
3. Establish a processing method that consistently results in a sample with a neutral pH.
4. Use no more than the recommended 0.5 mL MAS per bottle.

These steps, in combination with the other technical tips listed in this document will help to standardize the specimen processing procedure across samples, provide better control of the NaOH concentration and sample neutralization step, and allow a uniform neutral final pH to be obtained for all samples. This will minimize the BacT/ALERT® MP culture bottle false positive rate while helping to maximize Mycobacterium recovery rates.

EXERCISE SESSION (See Appendix I for discussion)

The following techniques enhance Time to Detection by increasing the numbers of viable mycobacteria recovered from a sample.

1. Proper centrifugation at 3000 xg
2. Immediate decantation after centrifugation
3. Resuspension of centrifuged pellet with <2 ml of phosphate buffer
4. Final pH of pellet between 6.8 and 7.5
5. Use of ONLY 0.5 mL of MAS
6. DO NOT attempt to concentrate MAS by using < 10 mL of reconstitution fluid!

[9] CLSI. *Laboratory Detection and Identification of Mycobacteria*; Approved Guideline. CLSI document M48-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008/ Appendix b.

The following techniques <u>help to control breakthrough contamination</u>.

1. Transport specimens to lab promptly and refrigerate immediately
2. Use of proper sample volume
3. Use of NACL-NaOH (working concentration 2%) processing reagent
4. Complete vortexing, 20-30 seconds, with inversion and use of a timer
5. Incubating 15 minutes at room temperature with use of a timer
6. Inoculation of tests in correct order: MP culture bottle, solid media, slide
7. DO NOT attempt to concentrate MAS by using < 10 mL of reconstitution fluid!

The following techniques <u>help prevent crossover contamination</u> between samples.

1. Use of individual pour tubes for reagents being added to test
2. Use of syringes or pipettes long enough to reach bottom of tube without touching sides of tube when sampling pellet
3. Careful recapping of centrifuge tube to include:
    a. Never having more than one tube top open at any given time
    b. Never allowing a tube cap to be placed sample side down on working surfaces.

TIPS to REMEMBER: "All currently available digesting/decontaminating agents are to some extent toxic to tubercle bacilli; therefore, to ensure the survival of the maximum number of bacilli in the specimen, the digestion/decontamination procedure must be precisely followed. In order for enough tubercle bacilli to survive to give a confirmatory diagnosis, it is inevitable that a proportion of cultures will be contaminated by other organisms…It is also important to note that a laboratory which experiences no contamination is probably using a method that kills too many of the tubercle bacilli."[10]

The generally accepted breakthrough contamination rate is 3-5% of specimens cultured on nonselective (solid) media. If the rate is less that 3%, the procedure is too harsh and might damage the mycobacteria.[11]

"The goal…is to inhibit the normal flora but not the hardier mycobacteria. It is important for the laboratory to monitor the overall rate of specimen contamination. The goal is not to reduce this rate to zero, since that would indicate too many mycobacteria are being lost in the decontamination process; instead, it is expected that under normal circumstances, between 2% and 5% of specimens will be overgrown by normal flora." [12]

[10] Kantor IN, Kim SJ, Frieden t, et al. 1998. Laboratory Services in Tuberculosis Control: Culture Part lll. WHO/TB/98.258. p 37
[11] Della-Latta P (ed): Mycobacteriology and Antimycobacterial Susceptibility Testing, in Isenberg HD (ed). *Clinical Microbiology Procedures Handbook*, vol 2. Washington, DC, ASM Press; 2004 sect 7.1.2.2
[12] CLSI. *Laboratory Detection and Identification of Mycobacteria*; Approved Guideline. CLSI document M48-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008

TECHNICAL PROCEDURE: MYCOBACTERIA SPECIMEN PROCESSING

SPECIMEN: The BacT/ALERT® MP System consists of the BacT/ALERT® MP culture bottle with a removable closure used in conjunction with the MB/BacT® Antibiotic Supplement (and/or the MB/BacT® Reconstitution Fluid). The BacT/ALERT® MP System is designed for use with the MB/BacT® or the BacT/ALERT® 3D Mycobacteria Detection Systems for recovery and detection of mycobacteria from sterile body specimens other than blood, and from digested-decontaminated clinical specimens.

REAGENTS:

BacT/ALERT® MP (color-coded red) – BacT/ALERT® MP disposable culture bottles with a removable closure contain 10 ml of media and an internal sensor that detects carbon dioxide as an indicator of microbial growth. The BacT/ALERT® MP culture bottle may be inoculated via a sterile syringe with either an attached locking needle or a sheathed needle or by removing the closure and using a needleless inoculation device.

The media formulation consists of: Middlebrook 7H9 Broth (0.47% w/v), Pancreatic Digest of Casein (0.1% w/v), Bovine Serum Albumin (1.0% w/v), Catalase (48 u/ml), in purified water. Bottles contain 10 ml of media, and are prepared with an atmosphere of carbon dioxide, nitrogen and oxygen under vacuum. The composition of the media may be adjusted to meet specific performance requirements.

MB/BacT® Antibiotic Supplement – Lyophilized supplement formulated to contain Amphotericin B (0.0180% w/v), Azlocillin (0.0034% w/v), Nalidixic Acid (0.0400% w/v), Polymyxin B (10,000 units), Trimethoprim (0.00105% w/v), Vancomycin (0.0005% w/v), and a bulking agent prior to processing. The composition of the supplement may be adjusted to meet specific performance requirements. Reconstitute with 10 ml of MB/BacT® Reconstitution Fluid.

NOTES:

- One vial of reconstituted MB/BacT® Antibiotic Supplement is sufficient for 20 BacT/ALERT® MP culture bottles.
- Once reconstituted, the MB/BacT® Antibiotic Supplement has a shelf life of 7 days when stored at 2-8°C. The expiration date for the reconstituted supplement should be recorded on the vial label.
- Remaining MB/BacT® Reconstitution Fluid may be stored at 2-8°C for later use with sterile specimens.

MB/BacT® Reconstitution Fluid – Oleic acid (0.05% w/v), Glycerol (5% w/v), Amaranth (0.004% w/v), and Bovine Serum Albumin (1% w/v) in purified water. Each bottle contains a total fill volume of 15 ml. The composition of the reconstitution fluid may be adjusted to meet specific performance requirements.

EQUIPMENT AND MATERIALS:

Additional materials required

Sterile distilled or deionized water
Middlebrook 7H11 or other mycobacterial agar or egg-base medium
Autoclave or other safe disposal method
N-Acetyl-L-Cysteine powder; decontamination reagents
Sterile 0.067 M phosphate buffer, pH 6.8
Centrifuge
CO_2 Incubator, 37°C

Sterile syringes with either attached locking needles or sheathed needles
Sterile pipettes or other sterile needleless inoculation device for bottles with removed closures
Mycobactericidal disinfectant
Alcohol swabs
Vortex mixer
Sterile 50 ml conical polypropylene centrifuge tubes
Biological safety cabinet
Sterile, disposable gloves
Disposable gowns
Disposable masks
Microscope
Materials for staining slides
Quality Control organisms

Materials available from bioMérieux
BacT/ALERT® 3D Mycobacteria Detection Systems
BacT/ALERT® MP culture bottles, PN 259797
MB/BacT® Antibiotic Supplement Kit, PN 259760
BacT/ALERT® Reseals, PN 259787 (to use removable closure system)

PROCEDURE:

1. Use no more than 10 ml of the specimen in a 50 ml conical centrifuge tube. Split larger samples into two or more aliquots and process the aliquots. Pellets may be combined at the end of the decontamination procedure for inoculation into one BacT/ALERT® MP Culture bottle.

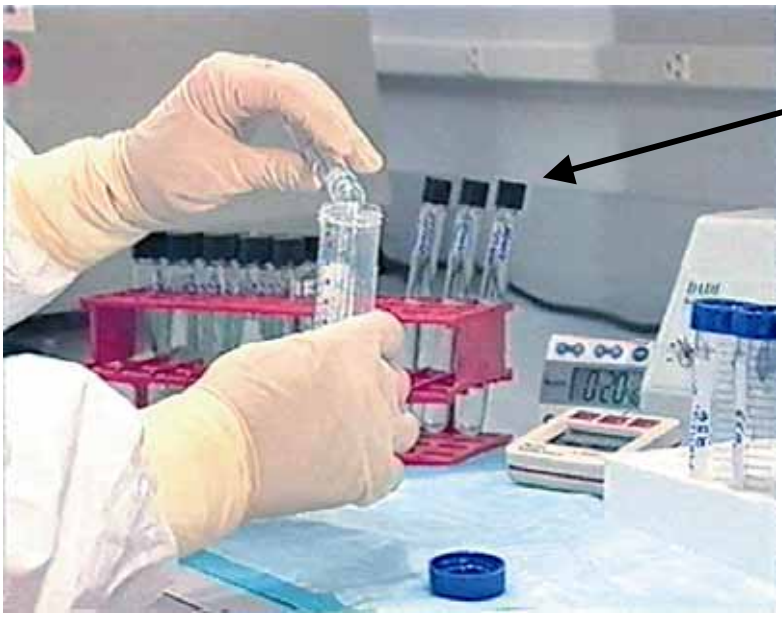
Assure that sample volumes used are less than or equal to 10 ml –

Split larger volumes into two or more tubes and process –

Add sterile saline to small volumes to standardize amount of NaOH added for decontamination and control final pH

✉**Note:** Splitting very mucoid samples may be accomplished more easily **after** adding an equal amount of decontamination/digestion reagent, vortexing and inverting 30 seconds. Pour equal amounts into **two or more** 50 ml conical centrifuge tubes. Process and combine same-sample sediments for inoculation of culture bottle, solid media and slide.

2. Add an equal volume of N-acetyl-L-cysteine (NALC)-Sodium Hydroxide (NaOH) solution to the specimen **from an individual pour tube.** Do not exceed 10 ml of NALC-NaOH per tube. Individual pour tubes prevent the addition of excess NaOH that will require pH neutralization later, and avoid the possibility of cross-contamination between specimens.



Use individual pour tubes for NALC-NaOH

3. Vortex for 20 seconds up to a maximum of 30 seconds. **Use a timer.** This breaks down the mucus. Be sure to **invert the tube** a few times during this process to insure that all areas of the specimen tube are decontaminated. Vortex gently because over foaming will cause NALC to oxidize and become inactive. Vortexing over 30 seconds may inactivate NALC. It may be necessary to add an extra pinch of NALC powder to very mucoid or bloody specimens to digest the specimen completely. If liquefaction is not complete during the initial vortex period, you may agitate the solution at intervals during step 4 to follow.

Use a timer for 20-30 seconds of vortexing

Incubate at room temperature for 15 minutes to decontaminate. **Use a timer.** **All** specimens (first to last) must incubate for 15 minutes. Sample batches that are too large (>20 samples) may cause incubation time to exceed 15 minutes and possibly delay time to detection. You may choose to invert and gently shake by hand at intervals during this incubation period.

Use a timer for 15 minutes RT incubation

4. Dilute specimen to a volume of 50 ml with **sterile** phosphate buffer **from an individual pour tube.** This reduces the action of the NaOH, which could injure the mycobacteria. Individual pour tubes avoid the possibility of cross-contamination between specimens.

Use individual pour tubes for Phosphate Buffer

5. **Mix by inverting** to be sure that all surfaces are coated and that the NaOH is neutralized. Refer to the nomogram provided on the next page and in Appendix F of this manual to determine the proper relative centrifugal force (RCF) using the centrifuge speed and radius.

Centrifuge for 15 minutes at 3000 x g (RCF), preferably at refrigerated temperatures, to increase cell viability and optimal recovery.

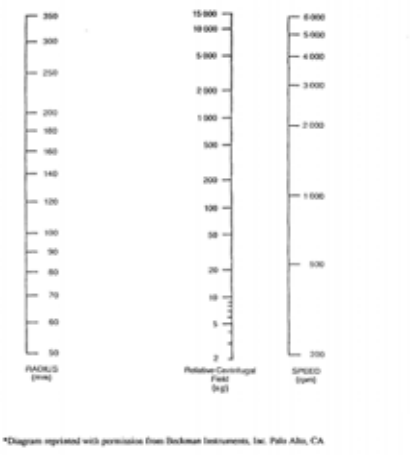


*Diagram reprinted with permission from Beckman Instruments, Inc. Palo Alto, CA

6. Immediately after centrifuge has stopped, pour off all supernatant completely into a splash-proof discard container filled with an appropriate tuberculocidal agent. Carefully wipe the lip of the tubes with **individual** gauze pads dampened with the disinfectant to clean up any drips – <u>avoid getting the disinfectant into the sample tube.</u>

7. Re-suspend the pellet in 1-2 ml of **sterile** phosphate buffer using **individual sterile transfer pipettes** to deliver the volume of buffer. Recap tube and mix pellet by vortexing.

Use individual sterile disposable transfer pipettes

✉ **Note:** It is recommended that you periodically check the final pH of the pellet with pH paper. The pH should be neutral between 6.8 and 7.5. A pH above 7.5 could be detrimental to the mycobacteria or possibly delay the time to detection. A pH lower than 6.8 may cause false positives.

Alternately, you may use reagents with phenol red added to the buffer so that neutral pH may be visualized when the resuspension buffer is added to the pellet after decantation.

INOCULATION OF BOTTLES, SLIDES AND SOLID MEDIA:

Use sterile technique during the following steps to avoid contaminating the culture bottles, MAS, and reconstitution fluid with environmental bacteria. The BacT/ALERT® MP culture bottles, MAS and reconstitution fluid should be at room temperature prior to mixing and inoculation.

✉ **Note:** Use individual disposable alcohol prep pads to clean the tops of all reagent or culture bottles before each entry.

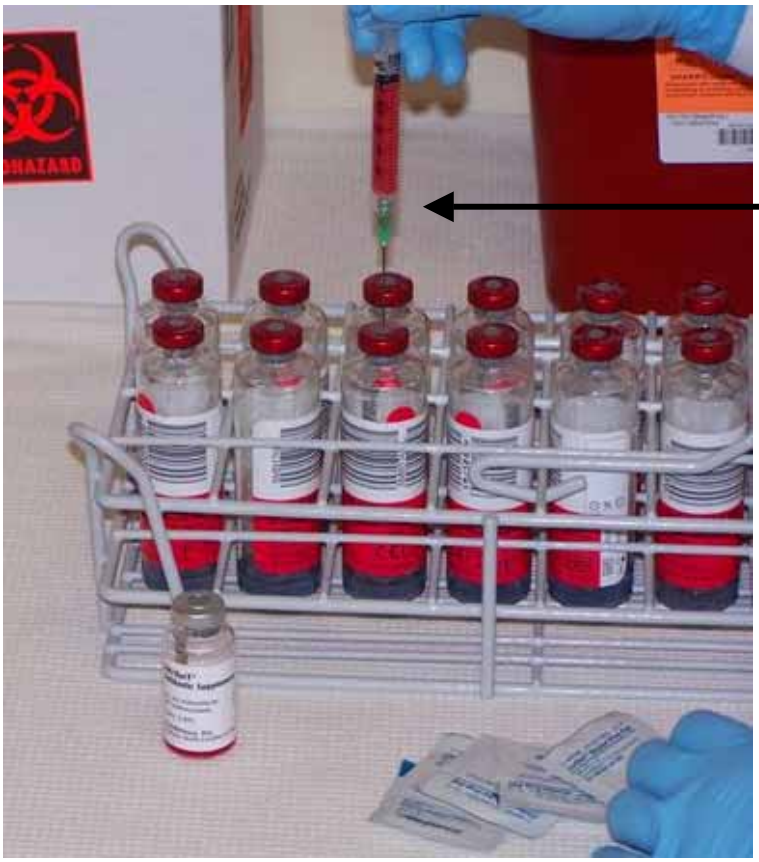
✉ **Note:** MAS may be pre-added to the BacT/ALERT® MP culture bottles and left in the refrigerator up to 24 hrs (within the limits of the expiration date of the MAS) before inoculation. Allow bottles to come to room temperature before using.

1. Label BacT/ALERT® MP culture bottles, solid media and slides appropriately. Do not cover bottle's barcode.
2. Reconstitute MB/BacT® Antibiotic Supplement with 10 ml of MB/BacT® Reconstitution Fluid using aseptic technique. Never re-use syringes.

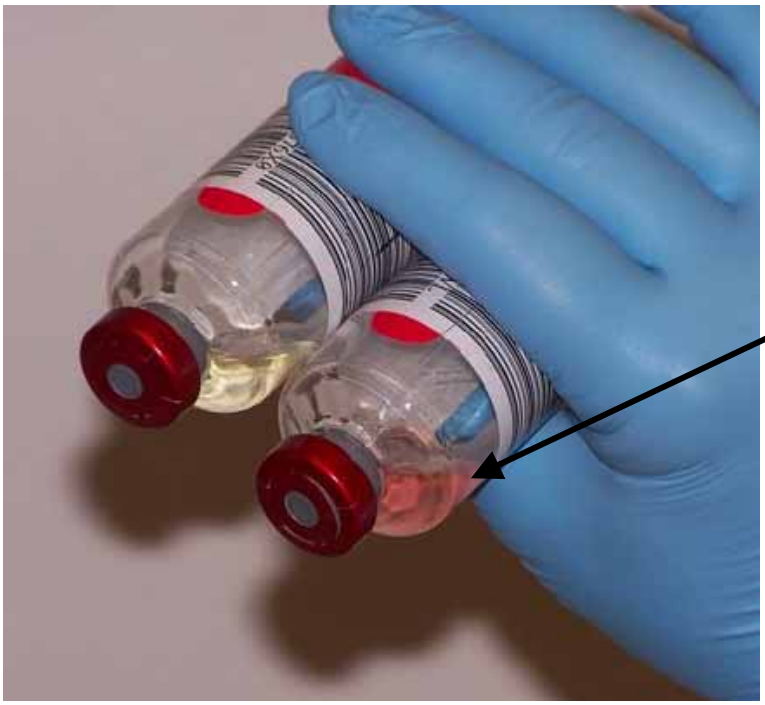
Aseptically withdraw 10 mL of Reconstitution Fluid

Aseptically add 10 mL of Reconstitution Fluid to Antibiotic Supplement vial and swirl to mix contents.

3. Add 0.5 ml reconstituted antibiotic supplement to each BacT/ALERT® MP culture bottle using aseptic technique for non-sterile samples. For sterile body fluids you may add 0.5 ml reconstitution fluid alone to the culture bottles. Antibiotics are added to lower breakthrough contamination of non-sterile specimens. Invert and gently swirl bottles to mix all contents.

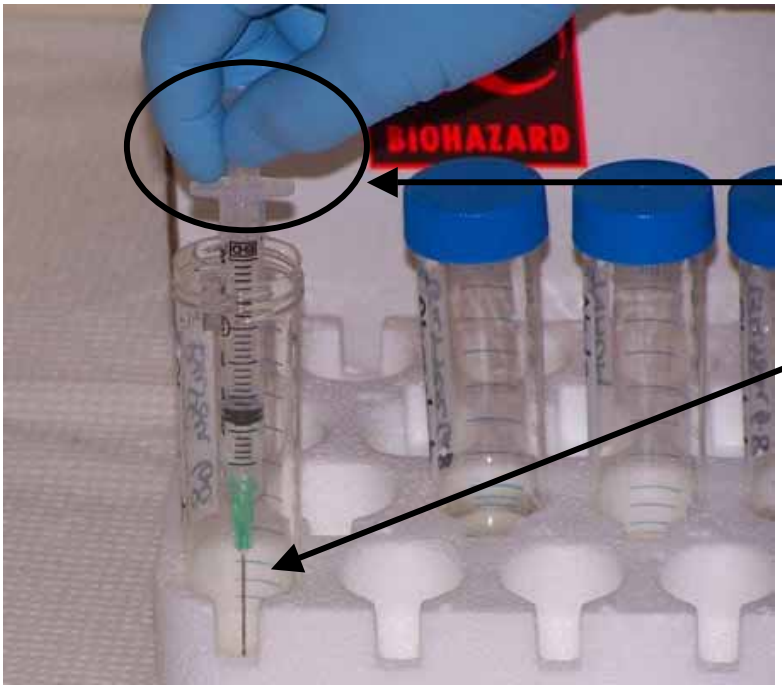


Add 0.5 mL reconstituted antibiotic supplement to each bottle for non-sterile specimens and 0.5 mL reconstitution fluid alone to bottles for sterile specimens.



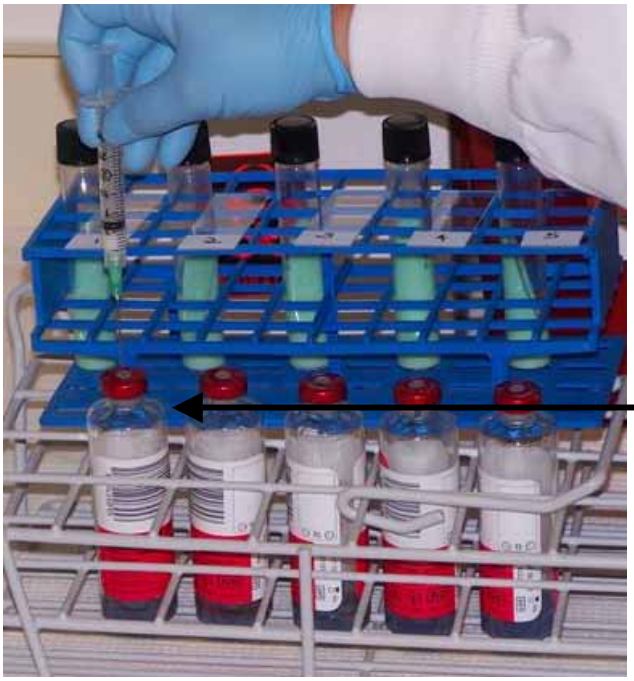
Do a "PINK CHECK" - The addition of this reagent is easily confirmed by visualizing a pink color in inoculated bottles

4. Under a biological safety cabinet, withdraw approximately 0.75 to 1.0 ml of the processed, resuspended specimen pellet using a sterile syringe with at least 1½ inch needle long enough to reach the bottom of the sample tube. This avoids contaminating the specimen by using smaller, more difficult to handle syringes. Use 18 to 22 gauge needles to prevent sampling errors due to small bore needles. (Alternatively, if bottle crimp and stopper are removed for needle-less inoculation, use one sterile transfer pipet per sample. (See page 34 of this manual.)



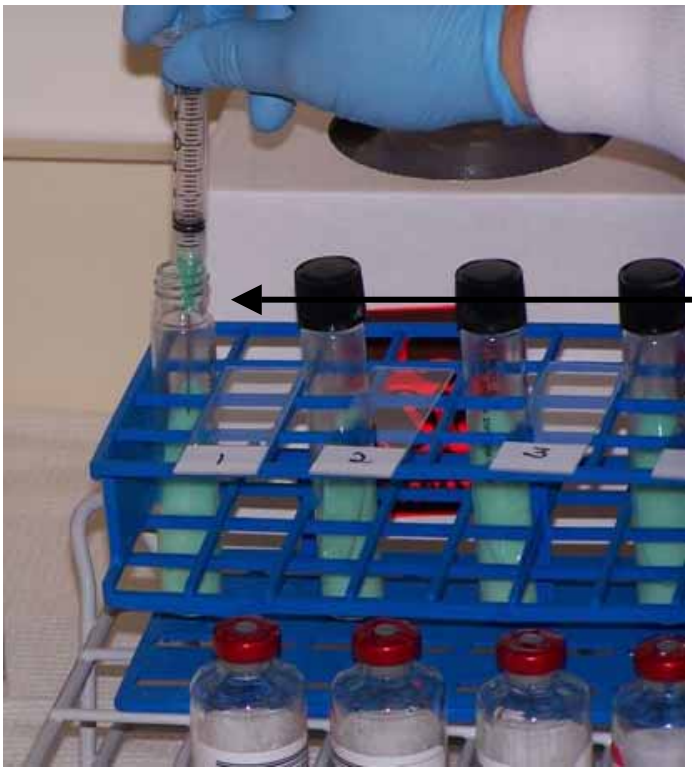
To avoid biohazard exposure to the operator or contamination of the sample, use syringes with needles long enough to reach the bottom of the tube.

5. First, add 0.5 ml of the sample to the appropriately labeled BacT/ALERT® MP culture bottle using aseptic technique.



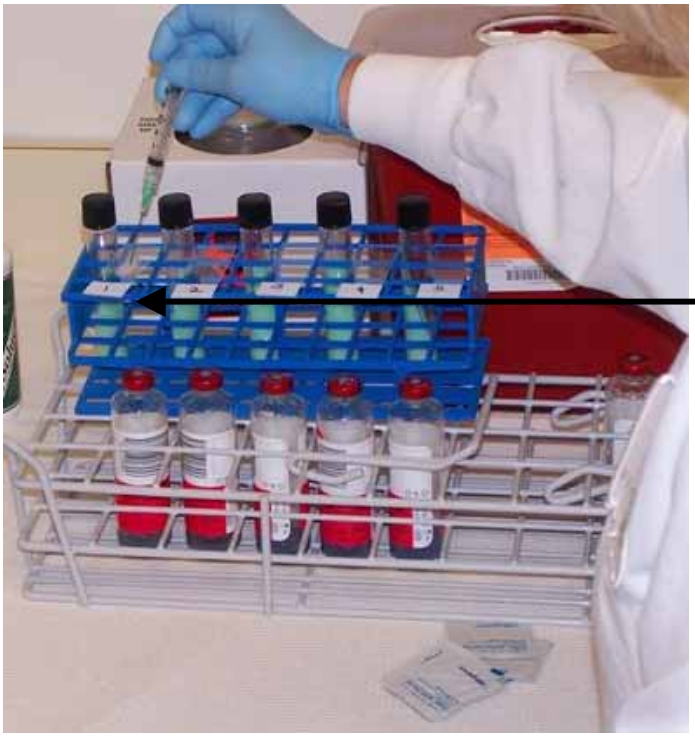
#1 – Sample into MP culture bottle

6. Second, using the same syringe (or pipet), inoculate appropriate solid media.



#2 – Sample to solid media

7. Third, place sample on slides (non-sterile) before discarding the syringe into a sharps (biohazard) container. Invert and gently swirl bottles to mix all reagents and sample.



#3 – Sample onto non-sterile slides

8. Clean tops of inoculated BacT/ALERT® MP culture bottles with a tuberculocidal agent (CAUTION: Cleaning agent must be compatible with polycarbonate plastic) prior to removal from the biological safety cabinet if they were inoculated with a needle. This removes any possible mycobacteria left on the tops of the bottles.

9. Load bottles into the red-handled MB drawers for BacT/ALERT® 3D instrument or into the MB/BacT® instrument. Refer to User Manual for assistance in loading. Default test day protocol holds bottles 42 days before reporting final negative result. Bottles should remain loaded for 42 days unless the instrument signals a positive.

NEEDLE-LESS INOCULATION OF BOTTLES, SLIDES AND SOLID MEDIA:

Perform this procedure under the biological safety cabinet. Take care to prevent contamination during both bottle preparation and inoculation of the patient sample. If aseptic needle-less inoculation is chosen, follow the instructions below after aseptically adding either the MAS or reconstitution fluid as described in this procedure.

Forceps

✉ **Caution:** Use care when removing the metal crimp seal. The use of forceps or other mechanical device is recommended. If the pull-tab breaks free from the seal resulting in sharp edges, never attempt to remove the seal by hand.

1. Remove the metal seal by pulling on the center tab across the top down through the rim of the seal. Avoid ripping the center tab completely away from the seal.

2. Continue to pull the tab around the bottle to remove the seal completely from the bottle.

3. Once the aluminum seal is removed, the rubber septum can be lifted off the bottle (with e.g. sterile forceps). The septum may be easier to remove if pried out of the bottle on either side of the septum slit. Special considerations are required to avoid contamination of the bottle.

4. Aseptically inoculate the specimen into the bottle via a needle-less device, such as a sterile disposable pipette.

5. Aseptically place the septum back into the bottle, ensuring that the septum fits fully into the opening. Place a reseal cap (BacT/ALERT® Reseal P/N 259787) over the septum and bottle opening. The reseal must catch under the bottle rim, and the underside of the reseal must be in full contact with the top of the septum. The reseal should fit tightly on the bottle with a “snap” sound.

6. This plastic reseal cap may be removed later for bottle sampling. First, vortex and invert bottle to mix contents. Remove reseal by pulling the tab and tearing it off.

FOR PLASTIC CULTURE BOTTLES:
BacT/ALERT® Reseal 100 units/box
P/N 259787

WHAT TO DO WITH A POSITIVE BacT/ALERT® MP CULTURE BOTTLE

Test procedure (adapted from Instructions for Use)

1. Load inoculated BacT/ALERT® MP culture bottles into the MB/BacT® or BacT/ALERT® 3D instrument following the instructions provided in the appropriate User Manual.

2. After culture bottles are loaded into the instrument, they should remain there for 42 days or until designated positive.

3. When the instrument indicates a positive bottle, remove the bottle according to procedures stated in the appropriate BacT/ALERT® User Manual.

NOTE: To reduce sampling error and enhance smear interpretation, proper mixing of a positive BacT/ALERT® MP culture bottle may be achieved by vortexing (up to 30 seconds) to break up any clumping present within the bottle. This must be done in a biological safety cabinet, using personal protective equipment.

4. All bottles designated positive should be smeared and subcultured for acid-fast bacilli. Subculturing may be performed by removing the sample with a needle and syringe from bottles without a Reseal or from bottles with a Reseal.

    a. **Subculturing from positive bottles without a Reseal using a needle and syringe.** In a biological safety cabinet, mix bottle contents, disinfect the bottle stopper, and remove specimen for acid-fast staining and subculturing. Disinfect bottle stopper after subculture.
    b. **Subculturing from positive bottles sealed with the Reseal.** Pull the center tab away from the Reseal and remove the seal completely from the bottle. After the septum is removed aseptically, the sample may be removed. If it is desired to keep the bottle for further testing, aseptically replace the stopper and place a new Reseal over the stopper and bottle.

    If the acid-fast smear is positive, proceed with the Mycobacteria specific identification procedures employed by your institution. If the concentrated smear is negative for acid-fast bacilli, a Gram stain should be performed. If both the acid-fast smear and the Gram stain are negative, indicating a possible false positive, the bottle should be loaded back into the instrument until growth of subculture or redesignation as positive or negative. A positive bottle status will automatically revert to negative-to-date when reloaded into BacT/ALERT® detection systems. Cultures which were initially determined false positive and were redesignated positive, should be smeared and subcultured.

5. If non-mycobacterial organisms are seen on the Gram stain, reprocess entire bottle contents through another decontamination procedure and inoculate into a new BacT/ALERT® MP culture bottle, or discard and obtain another specimen for culture. If the new BacT/ALERT® MP culture bottle again grows non-mycobacterial organisms, discard and obtain a new specimen for culture.

6. Signal negative cultures at the maximum test time should be visually examined for turbidity. If contents of the bottle are turbid, aseptically obtain a sample for acid-fast staining and subculture according to your laboratory's protocol. Bottles not exhibiting turbidity may be discarded. Decontaminate all bottles prior to disposal.

EXERCISE SESSION

1. What specimens may be added to the BacT/ALERT® MP culture bottle?
2. What is the bioMérieux recommended processing/decontaminating reagent?
3. What is the optimal pH of the centrifuged pellet prior to adding to the BacT/ALERT® MP Culture Bottle?
4. What is the recommended time to test for BacT/ALERT® MP culture bottles?

TIPS to REMEMBER:

1. The following techniques enhance Time to Detection by increasing the numbers of viable mycobacteria recovered from a sample.

a. Proper centrifugation at 3000 xg
b. Immediate decantation after centrifugation
c. Resuspension of centrifuged pellet with <2 ml of phosphate buffer
d. Final pH of pellet between 6.8 and 7.5
e. Use of ONLY 0.5 mL of MAS

2. The following techniques help to control breakthrough contamination.

a. Use of proper sample volume
b. Use of NACL-NaOH (working concentration 2%) processing reagent
c. Complete vortexing, 20-30 seconds, with inversion and use of a timer
d. Incubating 15 minutes at room temperature with use of a timer
e. Inoculation of tests in correct order: MP culture bottle, solid media, slide

3. The following techniques help prevent crossover contamination between samples.

a. Use of individual pour tubes for reagents being added to test
b. Use of syringes or pipettes long enough to reach bottom of tube without touching sides of tube when sampling pellet
c. Careful recapping of centrifuge tube to include:
    1. Never having more than one tube top open at any given time.
    2. Never allowing a tube cap to be placed sample side down on the working surfaces.

RESOURCES:

1. BioMérieux, Inc., BacT/ALERT® MP Instructions for Use, July 2008
2. Della-Latta P (ed): Mycobacteriology and Antimycobacterial Susceptibility Testing, in Isenberg HD (ed). *Clinical Microbiology Procedures Handbook*, vol 2. Washington, DC, ASM Press; 2004 sect 7.
3. Pfyffer GE: *Mycobacterium*: General Characteristics, Laboratory Detection, and Staining Procedures, in Murray PR, Baron EJ, Pfaller MA, et al (eds): *Manual of Clinical Microbiology,* ed 9. Washington, DC, American Society for Microbiology, 2007.

BacT/ALERT® MB Culture Bottle Procedure

OBJECTIVES: To know about the BacT/ALERT® MB Culture Bottle and Procedure

DISCUSSION:

BacT/ALERT® MB culture bottles with the addition of MB/BacT® Enrichment Fluid, when used with the MB/BacT® Mycobacteria Detection System (non-shaking) and the BacT/ALERT® Microbial Detection System (shaking), is a non-selective culture medium for the qualitative culture and recovery of mycobacteria from blood specimens.

BacT/ALERT® MB culture bottles in combination with the MB/BacT® Enrichment Fluid are designed for the cultivation of *Mycobacterium sp.* commonly isolated from blood. The medium will support growth of other aerobic organisms, including yeast, fungi, and bacteria. This complete system includes a lytic agent (Saponin), Sodium polyanetholesulfonate (SPS) and other media supplements which eliminate the processing step, prevent clotting of blood, and enhance the growth of mycobacteria.

A 3-5 ml volume of blood can be inoculated directly into the BacT/ALERT® MB culture bottle. Inoculated bottles are placed into the instrument where they are incubated (35°C-37°C) and continuously monitored for microbial growth.

REAGENTS:

BacT/ALERT® MB
BacT/ALERT® MB (color-coded black) – BacT/ALERT® MB sterile, disposable culture bottles contain 29 ml of media and an internal sensor that detects carbon dioxide as an indicator of microbial growth. The media formulation consists of Middlebrook 7H9 Broth (0.47% w/v), Pancreatic Digest of Casein (0.1% w/v), Glycerol (1.0% w/v), Sodium polyanetholesulfonate (0.025% w/v), in purified water. Bottles contain an atmosphere of carbon dioxide in oxygen and nitrogen under vacuum. The composition of the media may be adjusted to meet specific performance requirements.

MB/BacT® Enrichment Fluid
MB/BacT® Enrichment Fluid vials contain a total fill volume of 5.5 ml each. MB/BacT® Enrichment Fluid consists of Bovine Serum Albumin (14.5% w/v), Sodium Chloride (2.5% w/v), Oleic Acid (0.174% w/v), Saponin (4.4% w/v), in purified water.

Additional materials required
MB/BacT® Mycobacteria Detection System or BacT/ALERT® 3D Microbial Detection System
Middlebrook 7H11 or other mycobacterial agar or egg-base medium
CO_2 incubator, 37°C ± 2°C
Sterile syringes and needles
Mycobactericidal disinfectant
Blood drawing apparatus
Biological safety cabinet
Sterile, disposable gloves
Disposable gowns
Disposable masks
Appropriate biohazard waste containers for materials contaminated with infectious agents
Quality Control organisms

SPECIMEN COLLECTION AND PREPARATION:

Correct specimen collection is extremely important when obtaining blood culture specimens. Refer to Manual of Clinical Microbiology[1] for proper specimen collection and transport procedures. Proper skin disinfection is an essential requirement to reduce the incidence of contamination. Blood collected in EDTA and coagulated blood are not acceptable. Direct inoculation of blood onto a solid medium is not recommended.[1] Refer to the BacT/ALERT® MB Culture Bottle Instructions for Use for recommended collection procedures. BioMérieux recommends that inoculated culture bottles be placed into the BacT/ALERT® Microbial Detection System as soon as possible after collection. Inoculated culture bottles delayed in entry should be maintained at room temperature until they can be loaded into the incubator.[2]

BOTTLE PREPARATION:

1. Label the culture bottle with patient information. The icons on the bottle label, (☺, #, 🕑), can be defined by the user.
2. Remove plastic flip-top from culture bottle and disinfect with an alcohol swab or equivalent. Allow to air dry.
3. Enrichment Fluid is provided separately and must be added to the blood culture bottle for growth of mycobacteria. This addition can be done up to 24 hours before specimen inoculation, or if specimen inoculation is carried out at bedside, Enrichment Fluid can be added up to 24 hours after receiving the inoculated bottles in the laboratory.

    - Remove the plastic flip-top from the Enrichment Fluid vial and disinfect with an alcohol swab or equivalent. Allow to air dry.
    - Aseptically add 1 ml of Enrichment Fluid to each BacT/ALERT® MB culture bottle.
    - Employ careful, aseptic technique to avoid contamination of the Enrichment Fluid and medium in the culture bottle.

    NOTE: If the Enrichment Fluid is added following inoculation of the blood specimen, then this step must be performed in a biological safety cabinet while wearing appropriate protective clothing to comply with safety standards set forth by CDC/NIH for Biosafety Level 3 guidelines.[3] To avoid cross contamination, use a new syringe for each bottle containing blood.

    Clean tops of inoculated BacT/ALERT® MB culture bottles with a tuberculocidal agent (CAUTION: must be compatible with polycarbonate plastic) after addition of blood and prior to removal from the biological safety cabinet. This removes any possible mycobacteria left on the tops of the bottles.

[1] Pfyffer GE, Brown-Elliott BA, and Wallace RJ, Jr.: Mycobacterium, in Murray PR, Baron EJ, Pfaller MA, et al (eds): *Manual of Clinical Microbiology*, ed 8. Washington, DC, American Society for Microbiology, 2003, p 543.
[2] BioMerieux, Package Insert, Jul 2008
[3] Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

DIRECT DRAW INOCULATION PROCEDURE:

1. Prior to inoculation, disinfect the culture bottle top with an alcohol swab or equivalent. Allow to air dry.
2. Collect the blood using a butterfly set and the BacT/ALERT® Blood Collection Adapter Cap and inoculate directly into the BacT/ALERT® MB culture bottle at the patient's bedside (3-5 ml per bottle). To prevent overinoculation, monitor the blood volume intake into the Culture Bottle, using the 5 ml incremental markings on the bottle label.
3. After inoculation, swab bottle tops with gauze soaked in 2% Amphyl® or other mycobacteriocidal agent and allow to air dry.
4. Transfer the inoculated culture bottle promptly to the testing laboratory.

BLOOD COLLECTION TUBE INOCULATION PROCEDURE:

1. Blood may also be collected in a sterile SPS (Sodium polyanetholesulfonate) or heparinized tube and inoculated into the BacT/ALERT® MB culture bottle in the laboratory. No prior processing of the specimen is required. Blood collected in EDTA is unacceptable since EDTA inhibits mycobacterial growth even in trace amounts.
2. Disinfect the blood collection tube top prior to blood collection with an alcohol swab or equivalent. Allow to air dry. Disinfect the BacT/ALERT® MB culture bottle top with an alcohol swab or equivalent. Allow the septa to air dry prior to inoculation of the blood specimen.
3. Using aseptic technique, remove 3-5 mls from the collection tube with a sterile syringe and inoculate into the culture bottle.
4. After inoculation, swab bottle tops with gauze soaked in 2% Amphyl® or other mycobacteriocidal agent and allow to air dry.

SYRINGE INOCULATION PROCEDURE:

1. Prior to inoculation, disinfect the BacT/ALERT® MB culture bottle top with an individual, disposable alcohol prep pad. Allow to air dry.
2. Using aseptic technique, draw 3 ml - 5 ml of blood without anticoagulant from the patient and inoculate directly into the culture bottle.
3. After inoculation, swab bottle tops with gauze soaked in 2% Amphyl® or other tuberculocidal agent and allow to air dry. (CAUTION: Cleaning agent must be compatible with polycarbonate plastic)

WHAT TO DO WITH A POSITIVE BacT/ALERT® MB CULTURE BOTTLE: (Adapted from the BacT/ALERT® MB Culture Bottle Instructions for Use)

CAUTION: General caution should be taken when subculturing positive culture bottles as they could have been overfilled or contain high gas-producing organisms. Positive culture bottles contents may be under increased internal pressure. Positive culture bottles should be transiently vented before staining or disposal to release any gas produced during microbial metabolism.

1. Load inoculated BacT/ALERT® MB culture bottles into the MB/BacT® Mycobacteria Detection System (non-shaking) or the BacT/ALERT® Microbial Detection System (shaking), following the instructions provided in the User Manual.
2. After BacT/ALERT® MB culture bottles are loaded into the instrument, they should remain there for at least 42 days or until designated positive. **The following procedures require the use of Biosafety Level 3 practices, containment equipment and facilities.**[4]
3. When the instrument indicates that a particular cell contains a positive bottle, remove the bottle according to procedures provided in the User's Manual.
4. All bottles designated positive should be smeared and subcultured for acid-fast bacilli. In a biological safety cabinet, mix bottle contents, disinfect the bottle stopper with an alcohol swab or equivalent, and allow to air dry. Remove specimen for acid-fast staining and subculture using a syringe and needle. Disinfect bottle stopper with mycobacteriocidal agent after subculture. If the acid-fast smear is positive, proceed with the Mycobacteria specific identification procedures employed by your institution. If the smear is negative for acid-fast bacilli, but reveals the presence of other microorganisms, a Gram stain should be performed. If both the acid-fast smear and the Gram stain are negative, indicating a possible false positive, the bottle should be reloaded into the instrument until growth of subculture or redesignation as positive or negative. Cultures which were initially determined false positive and were redesignated positive should be smeared and subcultured.
5. If non-mycobacterial organisms are seen on the Gram stain, obtain another specimen for culture, if desired. The possibility of bacterial septicemia should also be considered.
6. Negative cultures may be checked by smear and/or subcultured at some point prior to discarding as negative.
7. **Do not reuse BacT/ALERT® culture bottles.** Dispose of inoculated BacT/ALERT® culture bottles according to your laboratory protocol. Autoclaving and/or incinerating inoculated BacT/ALERT® bottles is appropriate.[4]

[4] Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

EXERCISE SESSION (See Appendix I for answers.)

1. What are three acceptable inoculation methods for the BacT/ALERT® MB culture bottle?
2. What is the purpose of adding the MB/BacT® Enrichment Fluid?
3. How long before inoculation or after inoculation may the MB/BacT® Enrichment Fluid be added?

TIPS to REMEMBER

You must add 1.0 ml of the MB/BacT® Enrichment fluid to each BacT/ALERT® MB Culture bottle because it contains saponin to lyse the cells and growth factors necessary for Mycobacterial growth.

Mycobacteria Support Manual Objectives: After completion of this support manual, the participant should be able to:

_____Describe theory and operation of the BacT/ALERT® 3D or MB/BacT® System
- _____how CO_2 production affects the bottle sensor
- _____use of MB/PROCESS and MB/BLOOD algorithms

_____Describe the bottle preparation procedure for the BacT/ALERT® MP culture bottle
- _____culture bottle and MB/BacT® Antibiotic Supplement Kit storage conditions
- _____reconstitution of the MB/BacT® Antibiotic Supplement
- _____sample size-0.5 ml
- _____crimp removal and use of reseal cap for needle-less inoculation
- _____loading BacT/ALERT® MP culture bottles only into BacT/ALERT® MB drawers/racks

_____Describe specimen NALC-NaOH decontamination/digestion procedure
- _____contaminated specimen transport, storage and preparation
- _____sterile specimen preparation
- _____bottle and reagent inoculation procedures
- _____describe best practices regarding specimen processing/set-up
- _____digestion/decontamination of samples
- _____working or final NaOH concentration should be at least 2%
- _____use timer for incubation and vortexing times
- _____final pellet pH should be neutral 6.8 to 7.5
- _____quality control of decontamination method
- _____technique related sources of contamination, false positives and cross-contamination
- _____mycobacteria requiring special growth requirements, additives or temperatures
- _____causes of delayed detection times/false negatives

_____Describe the bottle preparation procedure for the BacT/ALERT® MB culture bottle
- _____storage conditions- room temperature
- _____bottle preparation and addition of MB/BacT® Enrichment Fluid
- _____describe 3 methods of bottle inoculation
- _____sample size and fill volume is 3 to 5 ml
- _____how to handle bacterial growth in MB bottle
- _____never load anonymously
- _____loading BacT/ALERT® MB culture bottles into either shaking (BC) or non-shaking (MB) drawers/racks

_____Describe testing methods any additional QC
- _____setting up QC cultures in BacT/ALERT® MP and MB culture bottles
- _____setting up seeded cultures in BacT/ALERT® MP and MB culture bottles

_____Review of appendix sections completed

Technologist's Signature_____Date_____

APPENDIX B: Other References for Mycobacterial testing

1. Instructions for Use, bioMérieux Inc, BacT/ALERT® MP culture bottle product # 259797, also contains information for MB/BacT® Antibiotic Supplement Kit product # 259760, BacT/ALERT® Reseal product # 259787

2. Instructions for Use, bioMérieux Inc, BacT/ALERT® MB culture bottle product # 251011, also contains information for MB/BacT® Enrichment Fluid product # 259877

3. *Manual of Clinical Microbiology*, 9th edition, Murray, Baron, Jorgensen, Landry, Pfaller. ASM Press, Washington D.C. 2007.

4. *Public Health Mycobacteriology, A Guide for the Level III Laboratory*, US Department of Health and Human Services-Center for Disease Control, Atlanta, GA, 1985.

5. *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

6. Clinical and Laboratory Standards Institute (CLSI). *Laboratory Detection and Identification of Mycobacteria; Approved Guideline*. CLSI document M48-A (ISBN 1-56238-669-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2008.

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8. Garcia, L. (ed in chief) *Clinical Microbiology Procedures Handbook*, 2nd edition, 2007 update, Washington, DC, ASM Press; 2007.

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1. John A. Crump, David C. Tanner, Stanley Mirrett, Celeste M. McKnight, L. Barth Reller, Duke University Medical Center, Durham NC, Carolinas Medical Center, Charlotte, NC " C-46 Controlled Comparison of Bactec 13A, Bactec Myco/F Lytic, BacT/ALERT MB, and Isolator 10 Systems For Detection Of Mycobactermia" J. Clin. Microbiol. 2003; 41(5):1987-1990

2. T. Hong, WR Butler, F. Hollis, MM Floyd, SR Toney, Yi-Wei Tang, D. Steele, and RJ Leggiadro. “Characterization of a Novel Rapidly Growing *Mycobaqcterium species* Associaed with Sepsis. J. Clin, Microbiol. 2003, p. 5650-5653,

3. Ana Paula, S. Louro, Ken B. Waites, Ecaterina Georgescu, and William H. Benjamin, Jr. “Direct Identification of *Mycobacterium avium* Complex and *Mycobacterium gordonae* from MB/BacT bottles using AccuProbe” J. Clin. Microbiol. 2001 39: 570-573.

4. Claudio Scarparo, Paola Piccoli, Alessandra Rigon, Giulana Ruggiero, Domenico Nista, and Claudio Piersimoni “Direct Identification of Mycobacteria from MB/BacT Alert 3D Bottles: Comparative Evaluation of Two Commercial Probe Assays” J. Clin. Microbiol. 2001 39: 3222-3227.

5. Fernando Alcaide, Miguel Angel Benitez, Josep M. Escribo, and Rogelio Martin “Evaluation of the Bactec MGIT 960 and the MB/BacT Systems for Recovery of Mycobacteria from Clinical Specimens for Species Identification by DNA AccuProbe” J. Clin. Microbiol. 2000 38(1): 398-401.

6. William H. Benjamin, Jr., Ken B. Waites, Stephen A. Moser and Andreas Roggenkamp “The MB/BacT Is a Sensitive Method of Isolating *Mycobacterium tuberculosis* from Clinical Specimens in a Laboratory with a Low Rate of Isolation” J. Clin. Microbiol. 2000 38: 3133-3134.

7. Harris G, Rayner A, Blair J, Watt B. “Comparison of three isolation systems for the culture of mycobacteria from respiratory and non-respiratory samples” J Clin Pathol. 2000 Aug; 53(8):615-8.

8. Francesca Brunello, Flavio Favari, and Roberta Fontana “Comparison of the MB/BacT and Bactec 460 TB Systems for Recovery of Mycobacteria from Various Clinical Specimens” J. Clin. Microbiol. 1999 37: 1206-1209.

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11. W.H. Benjamin, Jr., K.B. Waites, A. Beverly, L. Gibbs, M. Waller, S. Nix, S.A. Moser, and M. Willert “Comparison of the MB/BacT System with a Revised Antibiotic Supplement Kit to the Bactec 460 System for Detection of Mycobacteria in Clinical Specimens” J. Clin. Microbiol. 1998 36(11): 3235-3238.

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2. ASM 2002, C.T. Upchurch, S.B. Florence, P.H. Gilligan, UNC Hospitals, Chapel Hill, NC “C-48. Comparison of Decontamination Methodologies When Utilizing MB BacT/ALERT® 3D Mycobacterium Detection System”

3. ICAAC[2] 2000, Poster 1616, Karen Rondomanski, N. Glover, N. Anderson, and Lee Borenstein, Olive View-UCLA Medical Center, Sylmoar, CA and Los Angeles County Public Health Laboratory, Los Angeles, CA “ Evaluation of the Bactec MGIT 960 and the BacT/ALERT® 3D Systems for Recovery of Mycobacteria from Clinical Specimens”

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5. ASM 1998, S.F. Tidwell, MT(ASCP), T. Clark, B.S. Arkansas Department of Health, Little Rock, AR “Reduction of Bacterial Contamination in MB/BacT Bottles from Organon-Teknika”

[1] American Society of Microbiology Annual Meeting
[2] Interscience Conference on Antimicrobial Agents and Chemotherapy

Suggested Daily Workflow

1. Read and log temperature of incubator(s)
2. Perform cell calibration if indicated with error 60.
3. Identify any anonymous bottles as indicated on the screen.
4. Print an “Unload Positive Report” and then Unload Positive BacT/ALERT® MB or MP culture bottles. Either scan each bottle or remove bottles in a batch from the cells without scanning. Work up per the laboratory protocol.
5. Print an “Unload Negative Report” and then Unload Negative BacT/ALERT® MB or MP culture bottles. Either scan each bottle or remove bottles in a batch from the cells without scanning.
6. Prepare new samples for entry into the system following media package insert instructions and the laboratory protocol. Affix the small bottle barcode label on laboratory worksheet, if desired.
7. Enter new data into the Data Management computer as required and Load Bottles. Print load report to check for data entry errors.
8. Perform manual backup, if desired, on the BacT/ALERT® 3D.
9. Print or view the problem log on the Data Management computer, if desired.

Reagent Preparation

Working Phosphate Buffer with Phenol Red Indicator

Procedure for inclusion of Phenol Red Indicator in Phosphate Buffer

✉ **Note:** This allows the user to visualize the pH of the pellet without actual pH paper testing. If the pH of the resuspended pellet is out of range it will appear pink and should be adjusted to neutral pH and become colorless.

1. 0.067 M (M/15) phosphate buffer (pH 6.8)

a. Stock alkaline buffer

Na_2HPO_4 (anhydrous)	9.47 g
distilled water	to 1,000 ml

b. Stock acid buffer

KH_2PO_4	9.07 g
distilled water	to 1,000 ml

Add dry buffer powders to separate 1,000 ml volumetric flasks. Add distilled water to the 1,000 ml mark in each flask. Combine equal volumes of stock alkaline and acid buffers. Check the pH of the working solution with a pH meter. Add small amounts of alkaline buffer to raise the pH or small amounts of acid buffer to lower it.

2. Phenol Red indicator

Phenol Red	1.0 g	**or:**	0.2 g
distilled water	to 500 ml		to 100 ml

Add dry powder to volumetric flask and add distilled water to the volume mark. Mix well.

3. Working Phosphate Buffer (pH 6.8) with Phenol Red Indicator

Add 14* ml of the Phenol Red indicator to 4000* ml Working Phosphate Buffer. **Sterilize at 121°C for 20 minutes.** The solution may be stored at room temperature, but refrigeration is recommended.

*This is really a color intensity judgment. Add more or less as desired.

✉ **Note:** Use the above solution to buffer the NALC-NaOH/specimen mixture and to re-suspend the processed centrifuged MB/BacT® pellet. Further neutralization of the re-suspended pellet, if required, is accomplished by the drop wise addition of sterile 2N HCL until the indicator becomes clear. The pH should be between 6.8 and 7.5.

Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 1 of this section and discussed in Section 2. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Biosafety Level 1 - is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

Biosafety Level 2 - builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Biosafety Level 3 - is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment.

Biosafety Level 4 - is required for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. Access to the laboratory is controlled by the laboratory supervisor in accordance with institutional policies.

TABLE I
SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Laboratory bench and sink required
2	• Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPEs*: • Laboratory coats; gloves; face protection as needed	BSL-1 plus: • Autoclave available
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents PPEs: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in the text

---

Agent Summary Statements

Agent: *Mycobacterium tuberculosis complex* - The *Mycobacterium tuberculosis* complex includes *M. tuberculosis, M. bovis*, *M. africanum,* and *M. microti* that cause tuberculosis in humans, and more recently recognized *M. caprae* and *M. pinnipedii* that have been isolated from animals. *M. tuberculosis* grows slowly, requiring three weeks for formation of colonies on solid media. The organism has a thick, lipid-rich cell wall that renders bacilli resistant to harsh treatments including alkali and detergents and allows them to stain acid-fast.

Containment Recommendations –

BSL-2 practices and procedures, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a BSC.

BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex…

Agent: *Mycobacterium spp. other than M. tuberculosis complex and M. Leprae* - More than 100 species of mycobacteria are recognized. These include both slowly growing and rapidly growing species. In the past, mycobacterial isolates that were not identified as *M. tuberculosis* complex were often called atypical mycobacteria, but these are now more commonly referred to as nontuberculous mycobacteria or mycobacteria other than

tuberculosis. Many of the species are common environmental organisms, and approximately 25 of them are associated with infections in humans. A number of additional species are associated with infections in immunocompromised persons, especially HIV-infected individuals. All of these species are considered opportunistic pathogens in humans and none are considered communicable. Mycobacteria are frequently isolated from clinical samples but may not be associated with disease. The most common types of infections and causes are:
1. pulmonary disease with a clinical presentation resembling tuberculosis caused by *M. kansasii, M. avium*, and *M. intracellulare;*
2. lymphadenitis associated with *M. avium* and *M. scrofulaceum;*
3. disseminated infections in immunocompromised individuals caused by *M. avium*;
4. skin ulcers and soft tissue wound infections including Buruli ulcer caused by *M. ulcerans,* swimming pool granuloma caused by *M. marinum* associated with exposure to organisms in fresh and salt water and fish tanks, and tissue infections resulting from trauma, surgical procedures, or injection of contaminated materials caused by *M. fortuitum, M. chelonei*, and *M. abscesens*.

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures of *Mycobacteria* spp. other than *M. tuberculosis* complex. Clinical specimens may also contain *M. tuberculosis* and care must be exercised to ensure the correct identification of cultures. Special caution should be exercised in handling *M. ulcerans* to avoid skin exposure.

NSF International (The National Sanitation Foundation) conducts tests on biological safety cabinets to ensure the products meet minimum standards for cabinet classifications devised by NSF.

- NSF Standards are reviewed every 5 years.
- Tests are conducted on cabinets submitted to NSF by the manufacturers.
- Products which meet these standards are certified by NSF.
- Tests on cabinets are repeated every 5 years.

New Standard for Biological Safety Cabinets

NSF/ANSI (National Sanitation Foundation/American National Standards Institute)

NSF/ANSI 49 – 2002 pertains to all models of Class II cabinets (Type A1, A2, B1, B2) and provides a series of specifications regarding:
• Design/construction
• Performance
• Installation recommendations
• Recommended microbiological decontamination procedure
• References and specifications pertinent to Class II Biosafety Cabinetry.

Class II Cabinet Types

New NSF Classification, Adopted 2002	Previous NSF Classification	General Description
A1	Class II, Type A	■ 70% air recirculated; 30% exhausted from a common plenum to the room; ■ 75FPM intake; ■ may have biologically contaminated positive pressure plenum
A2	Class II, Type A/B3	■ 70% air recirculated; 30% exhausted from a common plenum to the room; ■ 100FPM intake; ■ biologically contaminated plenum under negative pressure or surrounded by negative pressure
A2	Class II, Type B3	■ 70% air recirculated; 30% exhausted from a common plenum to a facility exhaust system; ■ 100FPM intake; ■ biologically contaminated plenum under negative pressure or surrounded by negative pressure
B1	Class II, Type B1	■ 40% air recirculated; 60% exhausted from cabinet; ■ exhaust air pulled through dedicated exhaust duct into facility exhaust system; ■ 100FPM intake ■ all biologically contaminated plenums are negative to the room or surrounded by negative pressure plenums

B2	Class, II Type B2	■ 0% air recirculated; 100% exhausted from cabinet ■ exhaust air pulled through dedicated exhaust duct into facility exhaust system; ■ 100FPM intake ■ all ducts and plenums are under negative pressure ■ all contaminated ducts are under negative pressure or surrounded by directly exhausted negative pressure ducts or plenums

References:

Biosafety in Microbiological and Biomedical Laboratories. U.S. Department of Health and Human Services Public Health Service. Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. 2007. U. S. Government Printing Office Washington, DC.

The Baker Company
P.O. Drawer E
161 Gatehouse Road
Sanford, Maine 04073 USA
800-992-2537
E-mail - bakercoATbakercoDOTcom

Nomogram to Define Relative Centrifugal Force (RCF) for a Centrifuge

The centrifugal forces at a given radius are a function of rpm's. This nomogram is used for determining the relative centrifugal force (RCF), the revolutions per minute (rpm), or the radius when only two of these three variables are known.*

To find the unknown value, draw a straight line using a ruler through the two known values. Read the unknown value from the point of intersection on the column corresponding to that variable.

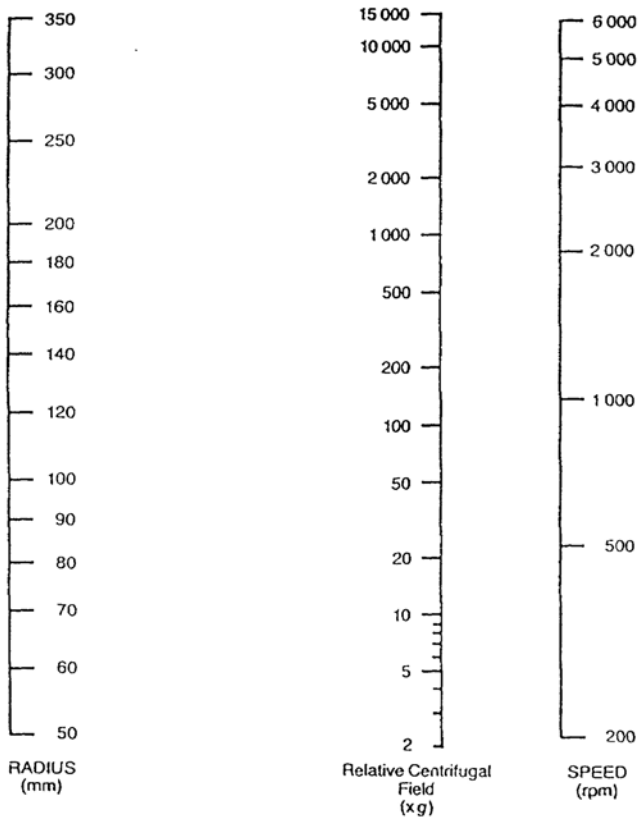


*Diagram reprinted with permission from Beckman Instruments, Inc. Palo Alto, CA

Clinical Study Data* **Comparison of BacT/ALERT® MP Culture Bottles and Conventional Methods for Recovery of all Mycobacteria[1]**

	Percent Sensitivity						
	TOTAL	MB	MB+LJ	MB+7H11	LJ+&H11	LJ	7H11
MAI	168.0	88.7	95.2	97.6	76.2	55.4	61.3
M. TB	183.0	87.4	93.4	96.7	89.6	72.1	64.5
M. chelonae	8.0	100.0	100.0	100.0	50.0	50.0	25.0
M. fortuitum	7.0	85.7	100.0	85.7	57.1	42.9	28.6
M. gordonae	28.0	100.0	100.0	100.0	17.9	17.9	0.0
M. kansasii	8.0	87.5	100.0	100.0	100.0	100.0	75.0
M. simiae	1.0	100.0	100.0	100.0	100.0	100.0	100.0
M. terrae	1.0	0.0	100.0	0.0	100.0	100.0	0.0
M. xenopi	1.0	100.0	100.0	100.0	100.0	100.0	100.0

The sensitivity of BacT/ALERT® MP culture bottles alone is 45% higher than LJ slants and 54% higher than 7H11 plates. BacT/ALERT® MP culture bottles show greater recovery of M. tuberculosis and the MAC group. BacT/ALERT® MP culture bottles also recover atypical mycobacteria such as M. chelonae and M. fortuitum in significantly greater numbers than solid media. See below and on next page that smear positive samples (greater numbers of organisms present in sample) have shorter time to detection and identification.

BacT/ALERT® Mean Time to Identification (Days)[1]

	Direct smear +	Direct smear --	Combo
MAI	9.5	14.8	13.0
M. TB	18.0	23.2	19.3
Other mycobacteria	13.6	21.4	18.7
All mycobacteria	15.4	18.6	16.7

*510(k) data on file at bioMérieux, Inc.

SEEDED STUDY AVERAGE TIME TO DETECTION[1]

Organism (no. of strains)	10^2 CFU/ml	10 CFU/ml
M. avium (4)	14.5	23.1
M. intracellulare (4)	15.1	25.2
M. tuberculosis (4)	20.6	22.6
M. kansasii	17.3	31.3
M. fortuitum	3.4	4.8
M. chelonae	9.3	22.3
M. xenopi	41.3	N.D.
M. bovis (2)	29.9	37.2
M. gordonae	14.2	17.1
M. simiae	10.1	12.9

[1] 510(k) data on file at bioMérieux, Inc.

BacT/ALERT® MP Instructions for Use 43-03064 July 2008

PERFORMANCE CHARACTERISTICS OF THE TEST

Seeded studies were performed using the following organisms at levels of ≤ 10^6 CFU/bottle and ≤ 10^2 CFU/bottle.

Microorganism	Inoculum (CFU/bottle)	Time to Detection (days)[1] BacT/ALERT MP (Plastic)
TB Complex		
M. tuberculosis (4 strains)	≤ 10^6	5.6 – 10.3
	≤ 10^2	13.7 – 18.8
M. bovis (2 strains)	≤ 10^6	7.1 – 7.8
	≤ 10^2	14.4 – 17.4
Photochromogens (Runyoun I)		
M. kansasii (2 strains)	≤ 10^6	7.4 – 8.8
	≤ 10^2	13.1 – 15.0
M. simiae	≤ 10^6	4.1
	≤ 10^2	8.9
Scotochromogens (Runyoun II)		
M. gordonae	≤ 10^6	14.1
	≤ 10^2	23.7
M. xenopi (2 strains)	≤ 10^6	10.3 – 13.8
	≤ 10^2	19.9 – 25.9[2]
M. scrofulaceum (2 strains)	≤ 10^6	12.6 – 23.3
	≤ 10^2	20.7 – NG[3]
Nonchromogens (Runyoun III)		
M. avium (4 strains)	≤ 10^6	4.2 – 6.8
	≤ 10^2	10.2 – 15.9
M. intracellulare (4 strains)	≤ 10^6	5.6 – 7.1
	≤ 10^2	10.8 – 16.2
M. malmoense	≤ 10^6	15.4
	≤ 10^2	21.9
Rapid Growers (Runyoun IV)		
M. chelonae	≤ 10^6	2.2
	≤ 10^2	4.1
M. fortuitum (2 strains)	≤ 10^6	3.5 – 4.1
	≤ 10^2	3.9 – 8.9

[1] Each organism was tested with MB/BacT Antibiotic Supplement. Testing was performed for single bottles at ≤ 10^6 and in triplicate at ≤ 10^2. Data listed represent a range of values from multiple strains, except for *M. simiae, M. gordonae, M. malmoense,* and *M. chelonae* where data for individual organism is given.
[2] One of three bottles showed no growth after 42 days; value given is average of two bottles.
[3] One organism tested showed no growth for three of three bottles after 42 days.

SPECIMEN PROCESS REAGENTS

SOURCES OF MISCELLANEOUS SUPPLIES:

NOTE: Many sources exist for the product types listed here. This manual shows a representative listing available from various websites but is not a complete list.

MYCOBACTERIOLOGY

- NAC-PAC™ EA3
- Digestion Systems
- Neutralizing Buffers
- Pellet Resusp. Buffer
- AFB Reagents
- AFB Stains
- Quality Control Slides
- CELL-BOND™ Slides

NAC-PAC™ Systems
NAC-PAC *Red*™

Alpha-Tec Systems, Inc. manufactures an array of reagents for effective specimen preparation for AFB testing that is available worldwide.

Alpha-Tec Systems, **Inc.**
P. O. Box 5435, Vancouver, WA USA 98662

For product inquiry and pricing:
USA phone: (800) 221-6058
International phone: (+) 360 260 2779
E-mail: CustSvc@AlphaTecSystems.com

Remel, Inc., produces NAC Attack (mucolytic agent is N-acetyl-L-cysteine) and Sputagest (mucolytic agent is Dithiothreitol) for AFB specimen preparation.

- NAC 100
- NAC 50
- NAC Attack
- Oxalic Acid
- Phosphate Buffer
- Sodium Citrate
- Sputagest 100
- Sputagest 50
- TB Base Digestant
- TB Digestant

Thermo Fisher Scientific
Remel Products
12076 Santa Fe Drive
P.O. Box 14428
Lenexa, KS USA 66215

USA Phone: (800) 255-6730
International Phone: (913) 888-0939
E-mail:
ICustomersupport@remel.com

N-acetyl-L-cysteine

NAC Attack

NAC Attack
REF: R210250
(Mucolytic Agent) For digestion and decontamination procedures for AFB cultures. Contents: 5 sealed ampules lyophilized N-acetyl-L-cysteine; 5 bottles TB Base Digestant.
5/Pk 50 ml/Bottle

NAC Attack
REF: R210500
(Mucolytic Agent) For digestion and decontamination procedures for AFB cultures. Contents: 5 sealed ampules lyophilized N-acetyl-L-cysteine; 5 bottles TB Base Digestant.
5/Pk 100 ml/Bottle

Cleland's Reagent – Dithiothreitol

Sputagest 50

Sputagest 50
REF: R21096
(Mucolytic Agent) Lyophilized, rehydrates to 50 ml. For digestion and decontamination procedures for AFB and fungal cultures. For use with 50 ml TB Base Digestant or Sodium Citrate.
6 Vials/Pk 50 ml/Vial

SALUBRIS, Inc.
GATEWAY TO HEALTH, WORLDWIDE

MYCOPROSAFE®

MycoProSafe® (MPS®) is a sample decontamination and concentration kit that permits the processing of samples for microscopy, culture and molecular methods for identification and isolation of mycobacteria. It is intended for in vitro diagnostic use. MPS provides, in individual sets, all the materials needed for processing each sample. It eliminates the problem of cross-contamination while saving time and effort. Stable pH (6.8-7.0). **Cat. #: MPS120 is available with 2% NaOH**

Headquarters and US office:
Baldwin Park I
12 Alfred Street, Suite 300
Woburn, Massachusetts 01801, U.S.A.
Phone (Toll Free): (800) 326-1484
Fax: (617) 249-0803

Europe:
16, avenue de Beauval
92380 Garches, France
Phone: +33 1 47 41 28 88

E-mail: info@salubrisinc.com

SDL Snap N' Digest®

Scientific Device Laboratory

NALC is supplied sealed in a glass ampule within a plastic bottle of NaOH/Sodium-Citrate solution. Squeeze the bottle to break the ampule when ready to use. Reagent stable for 24 hours after ampule broken. Product available in two sizes.
Kits also include packets of powder to make up sterile Phosphate Buffer, pH 6.8.

Cat No. 667, Snap N' Digest (75 ml, 10 bottles)
Cat No. 668, Snap N' Digest (150 ml, 10 bottles)
Cat No. 6671, Phosphate Buffer powder (5 packages of 4.2 gm each)
Cat 669-100, Sterile TB Buffer (pre-made Phosphate Buffer-10 bottles/100 ml)
Cat 669-500, Sterile TB Buffer (pre-made Phosphate Buffer-1 bottle 500 ml)

Scientific Device Laboratory, Inc.,
411 East Jarvis Avenue
Des Plaines, IL USA 60018
(847) 803-9495 for product inquiry and pricing or
www.scientificdevice.com

BD BBL® -MycoPrep®

BD

Mycobacteria Testing

Product Number: 240862

240862 - BD MycoPrep™ Specimen Digestion/Decontamination Kit Consists of 10 75 mL bottles of NALC-NaOH Solution and 5 packages of Phosphate Buffer (pH 6.8) (1 ea)

Key Product Features	
Availability	US, Canada, Mexico, Australia, New Zealand
Product type	BD BBL™ Specimen Collection and Processing
Brand	BD MycoPrep™

Mycobacteria Testing

Product Number: 240863

240863 - BD MycoPrep™ Specimen Digestion/Decontamination Kit Consists of 10 150 mL bottles of NALC-NaOH Solution and 10 packages of Phosphate Buffer (pH 6.8) (1 ea)

Maximum Time and Labor Savings When Compared with Other Reagent Preparation Protocols.

BD BBL™ MycoPrep™ Kit[1]	Typical TB Base Digestant and NAC-50 System[2]	Manual NALC-NaOH Method
1. Squeeze bottle to snap the NALC ampule. 2. Mix by gentle shaking. 3. Use as needed for up to 24 hours.	1. Allow NAC-50 vial to come to room temperature. 2. Transfer 5 mL of TB Base Digestant to NAC-50 vial. 3. Swirl to dissolve. 4. Add contents of NAC-50 vial to remaining 45 ml of TB Base Digestant. 5. Mix. 6. Use as needed for up to 24 hours.	1. Weigh 29 g of sodium citrate. 2. Add to 1 liter water. This is solution A. 3. Weigh 40 g of NaOH pellets. 4. Rapidly add to water, keeping water cool, to 1 liter. This is solution B. 5. Combine equal volumes of solutions A and B. 6. Sterilize (121°C for 15 min). This is NaOH-sodium citrate solution. 7. Divide and store in appropriate working volumes. 8. JUST BEFORE USE, weigh NALC to desired amount. 9. Add NALC to NaOH-sodium working volume. 10. Mix. 11. Use as needed for up to 24 hours.

NALC is supplied in an ampule within a plastic bottle of NaOH-Sodium Citrate solution. Squeeze the bottle to break the NALC ampule. Use within 24 hours. One packet of Phosphate Buffer makes 500 ml. Product is available in two sizes:

Product	Unit	Catalog No.
BBL™ MycoPrep™ Specimen Digestion/Decontamination Kit	10 75-mL bottles of NALC-NaOH Solution and 5 packages of Phosphate Buffer (pH 6.8)	240862
	10 150-mL bottles of NALC-NaOH Solution and 10 packages of Phosphate Buffer (pH 6.8)	240863

Becton Dickinson
1 Becton Drive
Franklin Lakes, NJ USA 07417
201 847 6800
www.BD.com

Hardy Diagnostics

HARDY DIAGNOSTICS
1430 W. McCoy Lane, Santa Maria, CA 93455
Phone: (805) 346-2766 ext.5658
Fax: (805) 346-2760
website: www.hardydiagnostics.com

DECONTAMINATION REAGENTS FOR THE RECOVERY OF MYCOBACTERIA FROM SPUTUM

Cat. no. U20	TB Base Digestant, 500ml Polypropylene Bottle, 500ml	500ml bottle
Cat. no. U22	TB Base Digestant, 125ml Polyethylene Bottle, 100ml	20 bottles/box
Cat. no. X45	TB Base Digestant, 2oz. Polyethylene Bottle, 50ml	25 bottles/box
Cat. no. Z60	N-Acetyl-L-Cysteine, 16x100mm Tube, 250mg	20 tubes/box
Cat. no. U10	Phosphate Buffer, 500ml Polypropylene Bottle, 500ml	500ml bottle
Cat. no. X43	Phosphate Buffer, 2oz. Polypropylene Bottle, 40ml	25 bottles/box
Cat. no. X31	Phosphate Buffer, 2oz. Polypropylene Bottle, 30ml	25 bottles/box
Cat. no. Z81	Bovine Serum Albumin, 13x100mm Tube, 3ml	20 tubes/box
Cat. no. Z163	N-Acetyl-L-Cysteine, 16x100mm Tube, 1.25gm	20 tubes/box
Cat. no. Z161	N-Acetyl-L-Cysteine, 16x100mm Tube, 2.5gm	20 tubes/box

STOCK CULTURES

ATCC® QC Strains - ATCC is the largest biological resource center in the world with the most comprehensive source of reference cultures.

The recommended "Seeded Study Protocol" strains are available directly from ATCC®. The current list price may be accessed at www.atcc.org. You may also contact ATCC directly at (800) 638-6597

American Type Culture Collection (ATCC)
P.O. Box 1549
Manassas, VA USA 20108
Tel: (703) 365-2700
(800) 638-6597 (U.S. and Puerto Rico)
Canadian customers contact CEDERLANE: (800) 268-5058 (North America)

- ***M. tuberculosis* ATCC 27294** (BTA MP culture bottle)
- ***M. intracellulare* ATCC 13950** (BTA MB & BTA MP culture bottles)
- ***M. kansasii* ATCC 12478** (BTA MP culture bottle)
- ***M. fortuitum* ATCC 6841** (BTA MP culture bottle)
- ***M. avium* ATCC 25291** (BTA MB culture bottle)

OTHER REAGENTS

Allied Monitor (660) 248-2823

- Ferric Mycobactin J – Product #62-0002 (2 mg)

Fisher Scientific Healthcare Products (800) 640-0640

- Ottowa Sand – 500 gm – Product # MSX00701

Sigma Aldrich Chemical Company, Inc. (800) 325-3010

- Hemin – Product #H9039 (Bovine

TISSUE GRINDERS

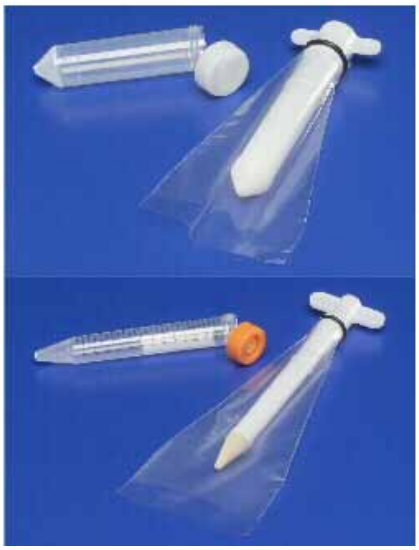
STERILE DISPOSABLE TISSUE GRINDER – AVAILABLE FROM MANY SOURCES…

tyco Healthcare
Kendall

Kendall PRECISION Disposable Tissue Grinder Systems

Features:

- Ready to use in sterile pouch.
- Completely disposable after grinding, no clean-up or sterilization required.
- Specimen can be cooled during grinding.
- Grinder tube doubles as a specimen container for transport and storage.
- Protective sleeve helps to contain any aerosolization created during grinding.
- Tissue can be ground quickly due to fresh grinding surface.



Grinders pictured here available from:
LifeLine Medical Inc.
22 Shelter Rock Lane
Danbury, CT 06810
(800) 452-4566 Toll Free
(203) 748-3806 Tel.

DIRECT DRAW BLOOD ADAPTOR

BacT/ALERT® Blood Collection Adapter for BTA MB culture bottle

–cap # 210361
–insert #210362

Kendall Healthcare (1-800-962-9888 www.kendallhq.com)

- Angel Wing® Blood Collection Device-Dome holder with Male Adapter and insert for BacT/ALERT® blood culture bottles – Product # 8881225245
- Angel Wing® Blood Transfer Device-Tube holder with Female Adapter and insert for BacT/ALERT® blood culture bottles – Product # 8881225241

SAFETY SYRINGES

Vanishpoint® Safety Syringes

Retractable Technologies, Inc.
511 Lobo Lane, P.O. Box 9
Little Elm, TX 75068-0009
Toll free: 888-806-2626
Phone: 972-294-1010

Becton Dickinson and Company (www.BD.com)

- **BD SAFETY-LOK ® Syringes –**

- **BD SAFETY-LOK® Syringes - (cont.)**
  309593 3 mL BD Safety-Lok® syringe with 22 G x 1 1/2 in. detachable needle, regular bevel, regular wall
  309595 3 mL BD Safety-Lok® syringe with 21 G x 1 1/2 in. detachable needle, regular bevel, regular wall

- **BD Integra™ Syringe with Retracting BD PrecisionGlide™ Needle**
  305272 22 G x 1 ½ in.
  305274 21 G x 1 ½ in.

QC SLIDES

KWIK-QC™ Slides provide the performance of QC and sample staining in a single step. Each slide has a positive and negative inoculated well and space for concurrent staining of culture isolates or clinical samples.

KWIK-QC™ Mycobacterium Slide	SL43-10

ALPHA-TEC

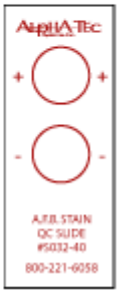
AFB QUALITY CONTROL SLIDES

AFB Stain Control Slides

Each slide contains an A.F.B. positive staining control of *Mycobacterium scrofulaceum* and an A.F.B. negative staining control of *Escherichia coli*.

AFB Stain Control Slides

10 slides/pk	032-43
40 slides/pk	032-40



Questions and Answers by Chapter

Chapter 1: Mycobacteria in our World

1. With 2 million deaths a year, what is the leading cause of death in many resource-poor and developing countries? The World Health Organization estimates that 9 million new cases and 2 million deaths are directly attributable to Tuberculosis and it has been cited as the leading cause of death in many resource-poor and developing countries.
2. What are MOTT? Mycobacteria other than the tubercle bacillus (MOTT) are also referred to as nontuberculosis mycobacteria (NTM).
3. Why is a solid media recommended when using liquid media culture systems? It is recommended that whenever possible, liquid medium should be used to assure better and more rapid results. The addition of solid medium along with a liquid medium maximizes the recovery, provides an opportunity to look at colony morphology that is not possible in liquid media, allows for detection of mixed mycobacterial infections and serves as a backup when liquid culture is contaminated. An increase of 4 to 6% for mycobacterial isolation has been reported by the addition of an LJ slant along with a liquid medium; while adding a liquid medium along with an LJ medium is reported to increase the recovery rate significantly (15 to 30%). The liquid-culture method is needed to provide more rapid detection, growth, and susceptibility results for *M. tuberculosis*.

Chapter 2: Theory of Operation

1. What metabolites were chosen to enhance CO_2 production during Mycobacterial metabolism? Once ingested, glycerol and oleic acid are converted to Acetyl-CoA and are oxidized through the Krebs or Tricarboxylic Acid Cycle (TCA). Carbon Dioxide (CO_2) and free electrons are the major metabolic byproducts of oxidation.
2. What technology is used to monitor the production of CO_2? The MB/BacT® and BacT/ALERT® 3D systems utilize a colorimetric sensor and reflected light to monitor the amount of CO_2 dissolved in the culture medium.
3. Why does the sensor change colors from dark to light? As CO_2 diffuses across the membrane and dissolves in the water contained in the sensor, free hydrogen ions are generated:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3$$

Free hydrogen ions interact with the indicator in the sensor. As CO_2 is produced, the concentration of hydrogen ions increases and the pH falls causing the sensor to change to lighter green or yellow.

Chapter 3: Media Overview – BacT/ALERT® MP Culture Bottle

1. How many BacT/ALERT® MP culture bottles are in one case? 100 bottles
2. What are the storage conditions for the BacT/ALERT® MP culture bottles and the MB/BacT® Antibiotic Supplement Kit? 2-8°C in dark
3. How many bottles can be prepared with one box of the MB/BacT® Antibiotic Supplement Kit? 100 bottles

Chapter 3: Media Overview – BacT/ALERT® MB Culture Bottle

1. How many BacT/ALERT® MB culture bottles are in one case? 25 bottles
2. What are the storage conditions for the MB Blood bottles and the MB/BacT® Enrichment Fluid? Room Temperature (15-30° C) in dark
3. How many bottles can be prepared with one box of MB/BacT® Enrichment Fluid? 25 bottles

Chapter 4: Best Practices in Sample Processing
Chapter 5: BacT/ALERT® MP Culture Bottle Technical Procedure

The following techniques enhance Time to Detection by increasing the numbers of viable mycobacteria recovered from a sample.

- ►Proper centrifugation at 3000 xg – Use the nomogram
- ►Immediate decantation after centrifugation
- ►Resuspension of centrifuged pellet with <2 ml of phosphate buffer
- ►Final pH of pellet between 6.8 and 7.5
- ►Use of ONLY 0.5 mL of MAS
- ►DO NOT attempt to concentrate MAS by using < 10 mL of reconstitution fluid!

The following techniques help to control breakthrough contamination.

- ►Transport specimens to lab promptly and refrigerate immediately
- ►Use of proper sample volume
- ►Use of NACL-NaOH (working concentration 2%) processing reagent
- ►Complete vortexing, 20-30 seconds, with inversion and use of a timer
- ►Incubating 15 minutes at room temperature with use of a timer
- ►Inoculation of tests in correct order: MP culture bottle, solid media, slide
- ►DO NOT attempt to concentrate MAS by using < 10 mL of reconstitution fluid!

The following techniques help prevent crossover contamination between samples.

- ►Use of individual pour tubes for reagents being added to test
- ►Use of syringes or pipettes long enough to reach bottom of tube without touching sides of tube when sampling pellet
- ►Careful recapping of centrifuge tube to include:
  - ●Never having more than one tube top open at any given time
  - ●Never allowing a tube cap to be placed sample side down on working surfaces.

Chapter 6: BacT/ALERT® MB Culture Bottle Technical Procedure

1. What are three acceptable inoculation methods for the BacT/ALERT® MB culture bottle? 1/Collect the blood using a butterfly set and the BacT/ALERT® Blood Collection Adapter Cap and inoculate directly into the BacT/ALERT® MB culture bottle at the patient's bedside (3-5 ml per bottle). 2/Blood may be collected in a sterile SPS or heparinized tube and inoculated into the BacT/ALERT® MB culture bottle in the laboratory. 3/Syringe draw 3 ml - 5 ml of blood without anticoagulant from the patient and inoculate directly into the blood culture bottle.
2. What is the purpose of adding the MB/BacT® Enrichment Fluid? MB/BacT® Enrichment Fluid is provided separately and must be added to the blood culture bottle for growth of mycobacteria. The enrichment fluid includes a lytic agent (Saponin), Sodium polyanetholesulfonate (SPS) and other media supplements which eliminate the processing step, prevent clotting of blood, and enhance the growth of mycobacteria.
3. How long before inoculation or after inoculation may the MB/BacT® Enrichment Fluid be added? Enrichment Fluid is provided separately and must be added to the blood culture bottle for growth of mycobacteria. This addition can be done within 24 hours of specimen inoculation, or if specimen inoculation is carried out at bedside, enrichment fluid can be added after receiving the inoculated vials in the laboratory.

BacT/ALERT® Media Quality Control

The following information is provided from Clinical Laboratory and Standards Institute (CLSI®) documentation as listed in appropriate footnotes:

"QC is an important integral part of laboratory testing. Laboratories should establish a protocol of all the aspects of QC testing, some of which are described below. The frequency of the QC testing depends upon the workload in a laboratory, type of media used, and laboratory-established policies.

All QC procedures should be written and results of QC testing documented in the laboratory records. In case there is a situation of unsatisfactory performance, corrective measures should be taken and their outcome should be documented. Records of batch numbers and expiration dates of all media and reagents used should be documented. QC records of commercial media may be obtained from the manufacturer and should be kept on file. QC should be carried out on culture media and reagents. Quality of laboratory procedures may also be evaluated by establishing normal averages of different parameters of results and then monitoring these parameters on specified time intervals.

Laboratory-prepared media should be thoroughly quality controlled. Inoculate 1% to 3% of each batch of newly prepared medium with a standardized suspension of at least three mycobacterial species (see below). Identify a previously prepared good lot of medium or a commercially prepared medium as a positive control. Using this medium, establish criteria for optimal growth to be obtained with a standardized inoculum and incubation period. The number and size of colonies within a specified time of incubation should be critically evaluated. If a newly prepared batch (lot) of medium, when tested, yields results below the established range, it should be considered unsatisfactory; colony counts 20% above or below the established range may be considered as acceptable, or laboratories may establish their own criteria. If the performance of the test medium is better than the control medium, this medium should be saved for the future QC control medium only after confirmation of its performance with repeat testing. This QC testing should be carried out with each newly prepared batch of medium. For commercially prepared media, follow the recommended procedures and criteria of performance. The following three mycobacterial cultures are recommended for QC testing:

M. tuberculosis, H37Ra	ATCC® 25177
M. kansasii	ATCC® 12478
M. fortuitum	ATCC® 6841

Commercially prepared media are thoroughly QC tested by the manufacturer. However, users should refer to CLSI®/NCCLS document M22 for media that are exempt/nonexempt from user QC. It is also important to visually examine commercially prepared media before use to make sure the tubes are not damaged/cracked, and that the medium has not been contaminated or changed in its appearance during transport or storage. For high-volume and reference laboratories, it is permissible to carry out QC testing of a new batch of commercial medium or reagents and document results. Follow the QC

procedures and criteria recommended by the manufacturers."[1]

The following information is from bioMérieux BacT/ALERT® MP and MB Culture Bottle Instructions for Use:

BacT/ALERT® MP culture bottles - A Certificate of Conformance is provided with each case of BacT/ALERT® MP culture bottles indicating satisfactory growth performance of *M. tuberculosis* ATCC® 25177 and *M. intracellulare* ATCC®13950. Upon receipt, new lots or shipments of BacT/ALERT® MP culture bottles or reagents may be tested for quality control. Refer to CLSI®/NCCLS M22-A3 for appropriate Quality Control organisms. Follow the Bottle preparation and inoculation procedure:

1. Add 0.5 ml of rehydrated MB/BacT® Antibiotic Supplement to each BacT/ALERT® MP culture bottle required for testing.
2. Inoculate representative bottles with 0.5 ml of the control organisms, diluted to 10^4 CFU/ml in sterile, physiological saline or sterile, unsupplemented Middlebrook 7H9 Broth (approved lot).
3. After obtaining anticipated results, use remaining bottles for testing clinical specimens. If expected results are not obtained, contact bioMérieux Customer Service.[2]

BacT/ALERT® MB culture bottles - A Certificate of Conformance is provided with each case of BacT/ALERT® MB culture bottles indicating satisfactory growth performance of *M. avium* ATCC® 25291 and *M. intracellulare* ATCC® 13950. Upon receipt, new lots or shipments of BacT/ALERT® MB culture bottles or reagents may be tested for quality control. Refer to CLSI®/NCCLS M22-A3 for appropriate Quality Control organisms. Follow the bottle preparation and inoculation procedure:

1. Add 1.0 ml MB/BacT® Enrichment Fluid to each BacT/ALERT® MB culture bottle required for testing.
2. Inoculate representative bottles with 5.0 ml of the control organisms, diluted to 10^3 CFU/ml in sterile, defibrinated sheep blood. Subculture on Middlebrook 7H11 plates to determine viability and count.
3. After obtaining anticipated results, use remaining bottles for testing clinical specimens. If expected results are not obtained, contact bioMérieux Customer Service.[3]

CLSI® documentation is available to describe the proper management of control organisms. Working suspensions of QC organisms "may be frozen in small aliquots (1 mL to 2 mL) in appropriate tubes/vials at –70 °C ±10 °C. The frozen suspension may be used for six months to a year. Once thawed, the culture suspension should not be refrozen."[4]

[1] CLSI. *Laboratory Detection and Identification of Mycobacteria; Approved Guideline*. CLSI document M48-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008, pg 31.

[2] Biomerieux, Inc., BacT/ALERT MP Bottle Package Insert, July 2008

[3] Biomerieux, Inc., BacT/ALERT MB Bottle Package Insert, July 2008

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"Commercial media are divided into two additional categories—exempt and nonexempt—which are determined by quality control performance data collected by CAP."[5] Exempt media "includes commercial media documented to maintain consistent user performance with minimum variation and requires minimum quality control."[6] "Provided the manufacturer can certify acceptable performance using the QC isolates listed in the table below, automated AFB broths for detection of mycobacteria are considered exempt. The user is instructed to refer to the package insert for specific QC information."[7]

Because bioMérieux's current Certificate of Conformance does not include all organisms listed in the table below, the user may choose to perform additional QC testing prior to use.

Table 2. Manufacturers' Minimum Quality Control Requirements for Commercially Prepared Media[7]

Medium	Incubation Atmosphere, Length and Temperature	Control Organisms (ATCC® No.)[a]	Expected Results
Mycobacteria media (Lowenstein Jenson agar and Middlebrook) broth medium used for recovery of AFB are exempt from user quality control provided that manufacturers certify acceptable performance using the QC isolates listed.	CO_2, <21 days, 35 ºC	*M. tuberculosis* H37Ra (25177) *M. kansasii* Group I (12478) *M. scrofulaceum* Group II (19981) *M. intracellulare* Group III (13950) *M. fortuitum* Group IV (6841) *E. coli* (25922)	Growth Growth Growth – May be inhibited on selective media Growth – May be inhibited on selective media Growth Inhibition (partial to complete on selective media)

[a] ATCC is a registered trademank of the American Type Cuture Collection

[4] CLSI. *Laboratory Detection and Identification of Mycobacteria; Approved Guideline*. CLSI document M48-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008, pg 61.

[5] NCCLS. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition*. NCCLS document M22-A3 (ISBN 1-56238-536-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087- 1898 USA, 2004. pg 4

[6] NCCLS. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition*. NCCLS document M22-A3 (ISBN 1-56238-536-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087- 1898 USA, 2004. pg 5

[7] NCCLS. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition*. NCCLS document M22-A3 (ISBN 1-56238-536-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087- 1898 USA, 2004. pg 14,17

Procedure for preparation of appropriate QC dilutions (10^4 and 10^3)

1. Prepare a culture grown on Lowenstein-Jensen or Middlebrook 7H11 media in a sterile screw-cap tube containing Middlebrook 7H9 broth with sterile Ottawa Sand or glass beads. Place colonies in a screw-cap tube, tighten the cap and vortex 20-30 seconds, or until well dispersed. Allow the suspension to stand for 15 minutes to allow large particles to settle.

2. For *Mycobacterium fortuitum, intracellulare,* and *avium* isolates: Transfer the supernatant into sterile 7H9 broth and adjust to a McFarland 0.5 Standard (1 x 10^8 CFU/ml). Dilute this stock as follows:

   1.0 ml of the above in 9.0 ml 7H9 broth (10^7)
   1.0 ml of the above in 9.0 ml 7H9 broth (10^6)
   1.0 ml of the above in 9.0 ml 7H9 broth (10^5)
   1.0 ml of the above in 9.0 ml 7H9 broth (10^4) (working dilution for MP culture bottle) **Mycobacterium fortuitum.*
   1.0 ml of the above in 9.0 ml sterile defibrinated sheep blood (10^3) (working dilution for MB culture bottle) **Mycobacterium avium,* and *M. intracellulare*

3. For *Mycobacterium tuberculosis* and *kansasii* isolates:
   Transfer the supernatant into sterile 7H9 broth and adjust to a McFarland 0.5 Standard (1 x 10^7 CFU/ml). Dilute this stock as follows:

   1.0 ml of the above in 9.0 ml 7H9 broth (10^6)
   1.0 ml of the above in 9.0 ml 7H9 broth (10^5)
   1.0 ml of the above in 9.0 ml 7H9 broth (10^4) (working dilution for MP culture bottle) **Mycobacterium tuberculosis,* and *M. kansasii*